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Drilling Fluid Effects to Developmental Stages of the American Lobster

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Laboratory experiments were conducted to evaluate the impact of drilling operations for oil exploration on populations of the American lobster (Homarus americanus). The effects of used, whole drilling fluids on the larval stages of the lobster were assessed in continous flow bioassay experiments. The five drilling fluids tested were shown to vary markedly in their toxicity, with LC-50 values ranging from 74 ppm to >500 ppm. Sublethal exposures to drilling fluids resulted in reductions in growth rates, molting frequencies, respiration rates, feeding rates, and the scope for growth index values. Reduced O:N ratios and increased protein: lipid ratios demonstrated a change in the energetics of the larval obsters as a result of drilling fluid exposure.

Results show that it is primarily the chemical and not the physical features of drilling fluids that were responsible for the detrimental effects observed. The drilling fluids tested that had a diesel component were more toxic than those without this component, although direct correlations between percent diesel and relative toxicity could not be made. The phenol and metal content of the drilling fluids may have also contributed to their toxicity. Field dispersion studies on drilling fluids are discussed in terms of potential impact areas.

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FOREWORD

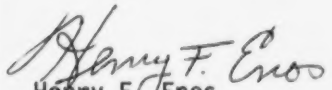
The protection of our estuarine and coastal areas from damage caused by toxic organic pollutants requires that regulations restricting the introduction of these compounds into the environment be formulated on a sound scientific basis. Accurate information describing dose-response relationships for organisms and ecosystems under varying conditions is required. The EPA Environmental Research Laboratory, Gulf Breeze, contributes to this information through research programs aimed at determining:

the effects of toxic organic pollutants on individual species and communities of organisms;

the effects of toxic organics on ecosystem processes and components;

the significance of chemical carcinogens in the estuarine and marine environments.

This report describes the toxicological and sublethal physiological responses of larval and postlarval stages of the American lobster to chronic exposure to drilling fluids produced from drilling operations for oil exploration. These data can be used to evaluate the impact of drilling operations for oil exploration on populations of the American lobster in the marine environment.



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ABSTRACT

Laboratory experiments were conducted to evaluate the impact of drilling operations for oil exploration on populations of the American lobster (Homarus americanus). The effects of used, whole drilling fluids on the larval stages of the lobster were assessed in continous flow bioassay experiments. The five drilling fluids tested were shown to vary markedly in their toxicity, with LC-50 values ranging from 74 ppm to >500 ppm. Sublethal exposures to drilling fluids resulted in reductions in growth rates, molting frequencies, respiration rates, feeding rates, and growth efficiencies. Reduced O:N ratios and increased protein:lipid ratios demonstrated a change in the energetics of the larval lobsters as a result of drilling fluid exposure.

Results show that it is primarily the chemical and not the physical features of drilling fluids that were responsible for the detrimental effects observed. The drilling fluids tested that had a diesel component were more toxic than those without this component, although direct correlations between percent diesel and relative toxicity could not be made. The phenol and metal content of the drilling fluids may have also contributed to their toxicity. Field dispersion studies on drilling fluids are discussed in terms of potential impact areas.

CONTENTS

ABSTRACT....iv
FIGURES....vi
TABLES....vii
ACKNOWLEDGEMENTS....x
1. INTRODUCTION....1
2. CONCLUSIONS AND RECOMMENDATIONS....4
3. MATERIALS AND METHODS....5
Culture of larval lobsters....5
Exposure system and techniques....5
Drilling fluids....7
Georges Bank sediment and barite....7
Physiological techniques....9
Feeding experiments....9
Biochemical analyses....9
Barium and calcium analyses....9
Statistical analyses....11
4. RESULTS....12
Physical and chemical characteristics of drilling fluids....12
Toxicity....12
Growth rates and molting....14
Respiration rates....20
Feeding rates....28
Ammonia excretion rates and O:N ratios....33
Biochemical composition....41
Barium and calcium composition....41
5. DISCUSSION....45
REFERENCES....49

FIGURES

Number Page

1. Schematic design of exposure apparatus.....6

2. Biochemical assay protocols.....10

3. Percent mortality at 96 h of control and drilling fluid exposed stage I lobster larvae.....15

4. Mean O:N ratios (± 1 S. E.) at 96 h of control and J-series drilling fluid exposed stage I lobster larvae.....38

TABLES

Number		Page
1.	Summary of toxicity tests	5
2.	Grain size analyses for drilling fluids	8
3.	Physical and chemical characteristics of the drilling fluids	13
4.	Four-day median lethal concentration (96-h LC_{50}) values (± 95 percent confidence limits) for drilling fluid exposed lobster larvae	14
5.	Percent survival at 96 h of control, Georges Bank-500 ppm and barite-500 ppm exposed stage I larvae	16
6.	ANOVA of percent survival comparison of stage I larvae	16
7.	Percent survival at 96 h of stage I larvae fed Artemia nauplii cultured in uncontaminated seawater, Georges Bank sediment and J-5 drilling fluids	16
8.	ANOVA of percent survival comparison of stage I larvae fed Artemia nauplii cultured in uncontaminated seawater, Georges Bank sediment and J-5 drilling fluid	16
9.	Mean dry weights (mg) of control and drilling fluid exposed stage I larvae at 24 h and 96 h	17
10.	ANOVA of mean dry weights of control and drilling fluid exposed stage I larvae at 24 h and 96 h	19
11.	Mean dry weights (mg) of control and J-4 drilling fluid exposed stage IV larvae at 96 h	21
12.	ANOVA of mean dry weights of control and J-4 drilling fluid exposed stage IV larvae at 96 h	21
13.	Mean dry weights (mg) of control and MB-5-29 drilling fluid exposed lobster larvae at 96 h	21
14.	Mean dry weights (mg) of control, Georges Bank-500 ppm exposed and barite-500 ppm exposed stage I larvae at 24 h and 96 h	21
15.	ANOVA of mean dry weights of control, Georges Bank-500 ppm exposed and barite-500 ppm exposed stage I larvae	22
16.	Mean dry weights (mg) at 96 h of stage I larvae fed Artemia nauplii cultured in uncontaminated seawater, Georges Bank sediment and J-5 drilling fluid	22
17.	ANOVA of mean dry weights at 96 h of stage I larvae fed Artemia nauplii cultured in uncontaminated seawater, Georges Bank sediment and J-5 drilling fluid	22
18.	Mean respiration rates (µl O_2/h) of control and drilling fluid exposed stage I larvae at 24 h and 96 h	23
19.	ANOVA of absolute respiration rates of control and drilling fluid exposed stage I larvae at 24 h and 96 h	24

Number Page

20. Mean weight-specific respiration rates (µl O_2/h/mg dry wt.) of control and drilling fluid exposed stage I larvae at 24 h and 96 h..25
21. ANOVA of weight-specific respiration rates of control and drilling fluid exposed stage I larvae at 24 h and 96 h.....26
22. Mean respiration rates (µl O_2/h) of control and J-4 drilling fluid exposed stage IV larvae.....27
23. ANOVA of absolute respiration rates of control and J-4 drilling fluid exposed stage IV.....27
24. Mean weight-specific respiration rates (µl O_2/h/mg dry wt.) of control and J-4 drilling fluid exposed stage IV larvae.....27
25. ANOVA of weight-specific respiration rates of control and J-4 drilling fluid exposed stage IV larvae.....28
26. Mean respiration rates (µl O_2/h) of control and MB-5-29 drilling fluid exposed stage I larvae.....29
27. ANOVA of absolute respiration rates of control and MB-5-29 drilling fluid exposed stage I larvae.....29
28. Mean weight-specific respiration rates (µl O_2/h/mg dry wt.) of control and MB-5-29 drilling fluid exposed stage I larvae.....29
29. ANOVA of weight-specific respiration rates of control and MB-5-29 drilling fluid exposed stage I larvae.....30
30. Mean respiration rates (µl O_2/h) at 24 h and 96 h of control, Georges Bank-500 ppm exposed and barite-500 ppm exposed stage I larvae.....30
31. ANOVA of absolute respiration rates of control, Georges Bank-500 ppm exposed and barite-500 ppm exposed stage I larvae.....30
32. Mean weight-specific respiration rates (µl O_2/h/mg dry wt.) at 24 h and 96 h of control Georges Bank-500 ppm exposed and barite-500 ppm exposed stage I larvae.....31
33. ANOVA of weight-specific respiration rates of control, Georges Bank-500 ppm exposed and barite-500 ppm exposed stage I larvae.....31
34. Mean respiration rates (µl O_2/h) at 96 h of stage I larvae fed Artemia nauplii cultured in uncontaminated seawater, Georges Bank sediment and J-5 drilling fluid.....31
35. ANOVA of absolute respiration rates of stage I larvae fed Artemia nauplii cultured in uncontaminated seawater, Georges Bank sediment and J-5 drilling fluid.....32
36. Mean weight-specific respiration rates (µl O_2/h/mg dry wt.) at 96 h of stage I larvae fed Artemia nauplii cultured in uncontaminated seawater, Georges Bank sediment and J-5 drilling fluid.....32
37. ANOVA of weight-specific respiration rates of stage I larvae fed Artemia nauplii cultured in uncontaminated seawater, Georges Bank sediment and J-5 drilling fluid.....32
38. Feeding rates of stage I larvae after 24 h exposure to 50 ppm J-5 drilling fluid.....33
39. ANOVA of feeding rates of stage I larvae after 24 h exposure to 50 ppm J-5 drilling fluid.....33
40. Growth efficiencies (K_1) for control and drilling fluid exposed stage I larvae at 24 h.....33

Number Page

41. Mean ammonia excretion rates and O:N ratios (± 1 S.E.) of control and drilling fluid exposed stage I larvae at 24 h and 96 h....34
42. ANOVA of ammonia excretion rates and O:N ratios of control and drilling fluid exposed stage I larvae at 24 h and 96 h....35
43. Mean ammonia excretion rates of control and J-4 drilling fluid exposed stage IV larvae....37
44. ANOVA of ammonia excretion rates of control and J-4 drilling fluid exposed stage IV larvae....37
45. Mean O:N ratios of control and J-4 drilling fluid exposed stage IV larvae....39
46. ANOVA of O:N ratios of control and J-4 drilling fluid exposed stage IV larvae....39
47. Mean ammonia excretion rates and O:N ratios (± 1 S.E.) of control, Georges Bank-500 ppm exposed and barite-500 ppm exposed stage I larvae at 96 h....40
48. ANOVA of ammonia excretion rates and O:N ratios of control, Georges Bank exposed and barite exposed stage I larvae....40
49. Mean ammonia excretion rates and O:N ratios (± 1 S.E.) at 96 h of stage I larvae fed *Artemia* nauplii cultured in uncontaminated seawater, Georges Bank sediment and J-5 drilling fluid....40
50. ANOVA of ammonia excretion rates and O:N ratios of stage I larvae fed *Artemia* nauplii cultured in uncontaminated seawater, Georges Bank sediment and J-5 drilling fluid....41
51. Biochemical analyses of control and drilling fluid exposed lobster larvae....42
52. ANOVA of biochemical analyses of control and drilling fluid exposed lobster larvae....43
53. Barium and calcium analyses of control, barite-500 ppm exposed and J-4 drilling fluid exposed stage I larvae at 96 h....44

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SECTION 1

INTRODUCTION

Interest in oil and gas exploration in the coastal regions of the northwestern Atlantic has increased in recent years in efforts to find petroleum resources. Increased exploitation, however, poses many unanswered questions concerning potential toxic effects of drilling and transport operations on commercially important species and the resulting economic impact on established fisheries.

The American lobster is found off the northeastern coast of the United States and off Atlantic Canada and is of particular ecological and economic importance. The life cycle of the lobster includes both planktonic and benthic stages (Herrick, 1896) and, thus, the lobster may be exposed to a wide range of stress conditions as a result of drilling operations. The lobster has four larval stages, the larval period extending from the time of hatching from the egg to the fifth molt or attainment of the first postlarval or juvenile stage (stage V). Stage IV larvae have fully developed claws and are active swimmers but may continue to be found among the plankton for several days before seeking a suitable benthic habitat (Cobb, 1976; Hughes and Matthiessen, 1962). Lobster populations in southern New England spawn during the early summer and the planktonic stages are abundant in surface waters from May through August at seawater temperatures of 15 to 24°C. Short-term exposure of these planktonic larval stages to pollutants discharged from drilling operations could result in reduced survival of larval lobsters, increased susceptibility of larvae to other environmental stresses and changes in the rates of larval growth and development. The impact of drilling activities and the toxicity of drilling fluids to lobster populations are poorly understood, but subtle effects on the physiology and behavior of lobsters may be expected with the release of drilling fluids to the marine environment.

Drilling fluids (or drilling muds) are complex mixtures of clays and chemical additives that are used in drilling operations to equalize hydrostatic pressure, remove drilled cuttings and lubricate the drill bit. The components of drilling fluids may include suspended solids, chromium compounds, alkaline compounds, bactericides, organic polymers, dispersants, defoamers, lubricants and detergents (Land, 1974). Different compounds are used to obtain various chemical and physical properties of the drilling fluids such as fluid density (barite), viscosity (ferrochrome lignosulfonate), corrosion control (paraformaldehyde), suspended load (bentonite) and lubrication (diesel oil), and the composition of drilling fluids may change during the various stages of the drilling operations (George, 1975).

These fluids and cuttings are continually discharged to the surrounding waters during the drilling operation and in bulk at the completion of an exploration or production well. A recent monitoring study of an offshore exploratory well in the mid-Atlantic determined that approximately 752 metric tons of barite, 1409 metric tons of low gravity solids (bentonite plus natural formation drill solids) and 94 metric tons of organic chemical components (chrome lignosulfonate, lignite and cellulose polymer) were discharged during a six-month drilling program (Ayers et al., 1980).

The dispersion of discharged drilling fluids and cuttings has been monitored in several field studies. In their study of discharges of drilling fluids in the Baltimore Canyon, Ayers et al. (1980) observed the formation of two plumes, a lower plume that contained solids such as cuttings and an upper plume that contained finer materials and remained as a diffuse cloud in the upper portion of the water column. Richards (1979) discussed field observations that indicate that near surface discharges of drilling fluids do not disperse uniformly but concentrate in surface waters above the thermocline. With these observations, the impact of drilling fluid exposure to planktonic organisms, including larval lobsters, becomes an important question.

Laboratory toxicity tests on the effects of drilling fluids to marine organisms have been generally limited to static bioassays with single components of drilling fluids (Daugherty, 1951; Cabrera, 1971; Chesser and McKenzie, 1975; and Rao et al., 1979). Toxic effects of the soluble components of drilling fluids (Thompson and Bright, 1977; Neff et al., 1981) and the suspended solids (EIFAC, 1965) have been demonstrated. In order to more closely approximate field conditions, continuous flow toxicity tests of used, whole drilling fluids should be investigated with a consideration of both lethal and sublethal effects.

Physiological and biochemical changes in an organism as a result of sublethal exposure to a toxicant can result in subsequent changes in growth and development and increased susceptibility to other environmental stresses (Capuzzo, 1977). Exposure of the grass shrimp Palaemonetes pugio to sublethal concentrations of pentachlorophenol, a drilling fluid bactericide, resulted in a decline in oxygen consumption and subsequent mortality (Rao et al., 1979). Brannon and Rao (1979) investigated the accumulation of barium, strontium and calcium by Palaemonetes with exposure to barite and found increased concentrations of barium in soft tissues and the exoskeleton of exposed shrimp. The ecological significance of these findings may be the increased transfer of barium in marine food chains. It has also been suggested that the disposal of drilling fluids may result in an increased barium content of seawater and sediments (Chow, 1976; Chow et al., 1978).

An understanding of the effects of drilling fluids on larval lobsters is needed to assess the impact of this marine pollutant on lobster populations and the lobster fishery. The objectives of our research have been to determine the comparative effects of various samples of used, whole drilling fluids on survival, growth and energetics of larval lobsters. The specific tasks of our research were as follows:

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(1) to compare the survival of larval lobsters exposed to various drilling fluids and to correlate toxicity with the chemical composition of drilling fluids;

(2) to assess the physiological effects of sublethal exposure to various drilling fluids on larval lobsters; and

(3) to assess alterations in biochemical and mineral composition of larval lobsters exposed to various drilling fluids.

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SECTION 2

CONCLUSIONS AND RECOMMENDATIONS

The present study examines the effects of whole, used drilling fluids on the larval stages of the American lobster. The different drilling fluids tested were shown to be highly variable in acute lethality and in their sublethal toxicity. The 96-h LC_{50} values for larval lobsters exposed to drilling fluids with a diesel component were among the lowest values thus far reported for a marine organism. Such a finding demonstrates that the choice of a sensitive toxicity test organism is very important in terms of providing an adequate evaluation of the toxicity of drilling fluids and their impact on the marine environment. This study also demonstrates the importance of testing whole, used drilling fluids in order to simulate as closely as possible the chemical pollutants that lobsters and other marine organisms will encounter with ocean discharges. In addition, chemical analysis of the drilling fluids being tested is necessary so that correlations between percent component content and relative toxicity can be determined.

A number of experimental variables should be controlled in toxicity tests evaluating drilling fluid toxicity. A flow-through toxicity test system is an effective means of exposing a test organism because the system allows for the removal of excretory products and for oxygen replacement. In a flow-through design, measures should be taken to provide a constant concentration of drilling fluid in the test chamber and to minimize loss of volatile components.

The effects of sublethal concentrations of drilling fluids should also be examined by monitoring physiological and biochemical changes of exposed organisms. Such a consideration can provide an indication of stressed condition and survival potential of exposed organisms.

The results of the present study also suggest the need for dispersion studies to determine the fate of the soluble components of drilling fluids, as these may often be the most toxic components. An understanding of the hydrodynamics of the area being drilled will also be necessary to predict the fate and effects of both the particulate and soluble phases of drilling fluid discharges.

SECTION 3

MATERIALS AND METHODS

Culture of larval lobsters

Female egg-bearing lobsters were obtained from local fishermen and maintained in flowing seawater (30-31 °/oo salinity) at ambient temperatures on a diet of mussels and squid. The eggs developed normally and began to hatch when seawater temperatures reached 18-20°C. After hatching, stage I larvae were transferred to fiberglass plankton-kreisels, described by Hughes et al. (1974), and maintained in flowing seawater at ambient temperatures. Tests conducted with stage I larvae began within 24 h after hatching. The later larval stages used in the toxicity tests were reared in the kreisels on a diet of frozen brine shrimp (Artemia sp.)

Exposure System and Techniques

Toxicity tests were conducted during three summers (Table 1). The

Table 1. Summary of toxicity tests.

Date	Drilling Fluids	Experiments
August 1979	MB-5-29	Stage I (Respiration experiments)
June-August 1980	MB-5-29; J-4	Stages I, II, III; Stage IV
May-August 1981	J-series	Stage I

larval lobsters were exposed to used, whole drilling fluids in a modification of the continuous-flow exposure system described by Capuzzo et al. (1976)(Fig. 1). In this system, seawater-pollutant mixtures were supplied by peristaltic pumping to thirty-two 500 ml fleakers(R) (Corning Glass) which served as test chambers. Modifications of the system were made to allow for a uniform distribution of a seawater drilling fluid mixture in the head-tanks and in the individual test chambers. The head-tanks (eight 13 l carboys) were supplied with magnetic stir-bars and placed on electric magnetic stirrers. Each test chamber held one stir bar and rested on a water-propelled magnetic stirrer. A false bottom of 1 mm Nitex(R) screening secured with clear, silicone sealant to the chamber floor prevented contact between the test organisms and the rotating stir bar. The test chambers were held in a water bath to maintain constant temperatures. Replacement time in each test chamber was estimated to be 99 percent replacement of

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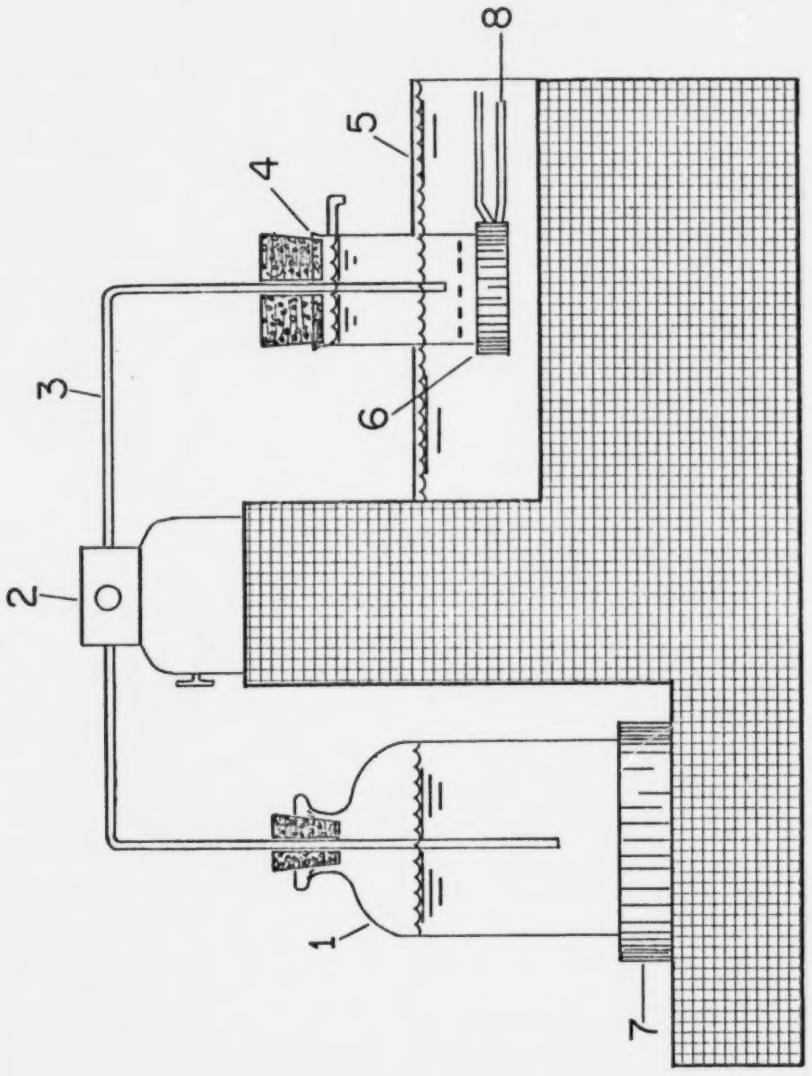

Figure 1. Schematic design of exposure apparatus - (1) 13-l seawater storage carboy; (2) peristaltic pump for seawater feed; (3) seawater supply line; (4) test chamber; (5) temperature controlled water bath; (6) magnetic stirrer for test chamber; (7) magnetic stirrer for seawater storage carboy; and (8) water line for magnetic stirrer.

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water in about 7.5 hours. This is well within the recommended range of flow-rates for toxicity testing (Sprague, 1969). Temperature, dissolved oxygen, and pH of the seawater and seawater-drilling fluid mixtures were monitored on a daily basis.

Larval lobsters were exposed for 96 h to concentrations of drilling fluids ranging from 1 to 500 ppm; four replicates at seven concentrations and a control were tested simultaneously. Lobsters exposed to filtered seawater and suspensions of barite and sediment collected from Georges Bank served as control experiments. Five larvae were added to each test chamber and maintained on a diet of live Artemia nauplii to reduce cannibalism. Larvae were monitored for survival, respiration rates, ammonia excretion rates and O:N ratios at selected times during the 96 h exposure period. Moribund larvae were examined microscopically; lack of heartbeat was the criterion of death. Control and exposed larvae were rinsed in distilled water and freeze-dried after the 96 h exposure period. Individual dry weights were determined with a Cahn Electrobalance.

A control experiment was conducted with stage I larval lobsters fed with drilling fluid exposed Artemia nauplii. Larvae were held in the continuous flow exposure system described above. Brazilian strain Artemia cysts were obtained from Aquafauna Inc. The Artemia hatching and exposure system consisted of a series of 500 ml glass separatory funnels, fitted with glass rods for gentle aeration. Artemia cysts were hatched and introduced to concentrations of J-5 drilling fluid ranging from 50 to 500 ppm for a 12 h period. Five lobster larvae were placed in the 500 ml test chambers and were supplied daily with 200 exposed nauplii per larva for 96 h. Respiration rates, ammonia excretion rates and O:N ratios were measured at 96 h and compared with control organisms maintained under identical conditions. Comparisons were also made on survival, individual dry weights and molt stage of control and experimental larvae.

Drilling Fluids

Used, whole drilling fluids were supplied by the U.S. Environmental Protection Agency, Gulf Breeze, Florida. The drilling fluids tested were water based lignosulfonate type muds. The J-series fluids (4 through 7) were collected in July 1980 from a land based well in Jay, Florida, at drilling depths of 12,257 ft., 13,399 ft., 13,997 ft. and 14,560 ft., respectively. The Mobile Bay drilling fluid collected 5-29-79 (MB-5-29) had been used in the Gulf of Mexico at drilling depths of about 14,500 ft. All drilling fluids were stored at 4°C.

The chemical characteristics of the J-series fluids were determined by Robert Shokes of Science Applications, Inc., La Jolla, CA. Information on the chemical composition of MB-5-29 was provided in Environmental Protection Agency reports submitted by Thomas R. Gilbert of the New England Aquarium, Boston, MA. and K. Ranga Rao of the University of West Florida, Pensacola, Florida. Phenol analyses were conducted by Energy Resources Co., Inc., Cambridge, MA, on all of the drilling fluids assayed. A grain size analysis, employing a Coulter particle size counter, was conducted for each drilling fluid and the Georges Bank sediment and barite. The percent distribution of clays, silts, and sands is presented in Table 2.

Table 2. Grain size analyses for drilling fluids.[a]

Percent Composition Drilling Fluid	Phi (ϕ) unit/diameter μ range								
	Clay			Silt				Sand	
	11ϕ/.5-.8	10ϕ/1.0-1.6	9ϕ/2.0-3.2	8ϕ/4.0-6.4	7ϕ/8.0-12.7	6ϕ/16.0-25.4	5ϕ/32.0-50.8	4ϕ/64.0	3ϕ
J-4	17.1	23.7	14.9	11.2	11.2	14.1	5.8	2.0	-
J-5	14.6	14.2	13.8	13.3	13.6	20.6	7.8	2.3	-
J-6	16.1	22.9	16.0	14.7	11.6	11.7	5.1	1.9	-
J-7	18.7	24.3	15.1	12.0	10.7	11.4	5.8	1.9	-
MB-5-29	9.2	13.9	13.0	20.7	21.4	16.0	6.4	1.0	-
Barite	9.1	8.8	12.4	15.7	18.1	18.7	12.3	3.4	1.5
Georges Bank Sediment	1.7	2.8	4.4	6.9	9.6	17.2	16.5	35.7	5.3

[a]Analyses performed by Mack Ferrebee, U.S. Geological Survey, Woods Hole, Massachusetts.

Georges Bank sediment and barite

The Georges Bank sediment was collected in an area of fine-grained sediment south of Martha's Vineyard, MA, called the Mud Patch. Samples were supplied by Rosemarie Petrecca and Frederick Grassle of the Woods Hole Oceanographic Institution, Woods Hole, MA. The Milbar brand barite samples were supplied by the Environmental Protection Agency, Gulf Breeze, FL.

Physiological techniques

Respiration rates of individual larvae were measured using a Gilson differential respirometer according to the techniques described by Capuzzo and Lancaster (1979). Equilibrium between the gas and liquid phase of O_2 diffusion in the respirometer flasks was enhanced by gently shaking the respirometer flasks. At the end of each set of oxygen uptake measurements, the seawater in the respirometer flasks was analyzed for NH_4^+-N by the method of Solorzano (1969) in order that an *in situ* estimate of ammonia excretion rates and the O:N ratio (atomic ratio of oxygen consumed to NH_4^+-N excreted) could be made; ammonia levels were compared with control blanks.

Feeding experiments

Food consumption of stage I larvae exposed to drilling fluid for 24 h was measured by adding a known quantity of *Artemia* nauplii and counting those remaining after 1 h. Individual larvae were tested in 100 ml glass vials with filtered seawater, Georges Bank sediment and drilling fluid seawater mixtures.

The calculations for growth efficiencies are presented in Table 40. Caloric determinations on freeze-dried *Artemia* nauplii were made on a Phillipson Microbomb Calorimeter; the caloric content was 4.4 calories·mg^{-1} and an individual nauplius weighed 1.48 µg.

Biochemical analyses

The relative percentages of protein, carbohydrate, lipid, and ash were determined for 96 h exposed and control lobsters. A flow chart of the protocol developed for these analyses is given in Figure 2. Freeze-dried larval lobsters were ashed at 475°C for 8 h in a muffle furnace. Protein extraction was accomplished with modification of methods previously described by Holland and Gabbott (1971). The Bio Rad protein dye binding assay was used to quantify protein concentrations colorimetrically (van Kley and Hale, 1977). Lipids were extracted by the combined methods of Bligh and Dyer (1959) and Folch et al. (1957) and quantified gravimetrically. The carbohydrate assay employed is described by Raymont et al. (1964).

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Figure 2. Biochemical assay protocols

Protein and Carbohydrate Assay Protocol-colorimetric.

Homogenate in 600 µl cold 5% TCA
(1-2 mg individual dried larvae/replicate)

Sonicate and store for 10 min at 4°C.
Centrifuge 10 min; collect supernatant
and wash precipitate with 400 µl 5% TCA.
Sonicate, centrifuge and combine supernatants.

Precipitate	Supernatant
Add 3 ml 0.1 N NaOH; sonicate and heat for 30 min at 80°C.	Test 400 µl samples (2 replicates). Add 600 µl 5% TCA to bring total to 1.0 ml. Add 1.0 ml 5% phenol, mix, and add 5.0 mls concentrated H_2SO_4, vortex. Read at 490 nm after 20 min.
Test 200 µl samples (3 replicates). Add 800 µl 0.1 N NaOH to bring total to 1.0 ml. Then add 1.0 ml 0.15 N HCl, stir. Add 0.5 ml BioRad reagent, vortex and read at 595 nm after 15 min.	
Protein	Total Carbohydrate

Lipid Assay Protocol-gravimetric.

Homogenate in 1.9 ml chloroform-methanol-water
(1:2:0.8) (3-5 mg dried larvae)

Sonicate and centrifuge for 10 min. Decant supernatant. Extract again with chloroform-methanol (2:1), sonicate and centrifuge. Collect and combine supernatants.

Wash with 0.95 ml 0.7% NaCl, vortex. Store for 30 min at 4°C; centrifuge 10 min. Discard top methanol-water layer.

Evaporate 3-300 µl samples of the chloroform-lipid layer in aluminum pans on an aluminum block heater at 50°C. Determine lipid weight minus pan weight.

Lipid

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PAGE NUMBER
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(4-76)

Barium and calcium analyses

Elemental analyses for Ba and Ca were carried out on 5.0–10.0 mg pooled samples of dried, agate mortar-ground, 96-h exposed and control stage I lobster larvae by means of instrumental neutron activation analysis. Irradiations were carried out at the Rhode Island Nuclear Science Center according to the techniques described by Spencer et al. (1978).

Statistical analyses

Statistical techniques were adopted from Sokal and Rohlf (1969). A priori comparisons of means and groups of means were analyzed by analysis of variance. As required by this test, the sum of the degrees of freedom of the separate a priori tests does not exceed the total number of groups being compared minus one.

Median lethal concentrations (LC_{50}) and confidence limits at 96 h were estimated by the BMD 03S revised program of probit analysis (Health Sciences Computing Facility, University of California at Los Angeles). The program allowed for log transformation of exposure concentrations and corrected for mortality in the controls (Finney, 1971).

SECTION 4

RESULTS

Drilling Fluids

The physical and chemical characteristics of the J-series and Mobile Bay drilling fluids are presented in Table 3. The J-drilling fluids ranged in water content from 80-88 percent. Of the metals analyzed in the J-series, lead (19 to 42 ppm), chromium (96 to 201 ppm), and zinc (175 to 271 ppm) were present in high concentrations. Total barium concentrations were also high (4 to 12 percent) as is typical of drilling fluids due to the major clay constituent, barite. Calcium concentrations ranged from 40 to 140 ppm. The percent diesel content for J-4, 5, 6 and 7 were 0, 2, 3 and 4, respectively. MB-5-29 had a No. 2 fuel oil concentration of 0.27 percent and a phenol concentration of 1.3 ppm. The chromium concentration of 1810.0 ppm reported for MB-5-29 was higher than the concentrations of any of the J-series drilling fluids.

Phenol concentrations were 1.3 ppm in MB-5-29, 0.45 ppm in J-5, 0.33 ppm in J-7, and below the 0.25 ppm detection limits in J-4 and J-6. Due to their high toxicity, halogenated phenols have been banned from use as biocide additives to drilling fluids used offshore since 1979 (44 Fed. Reg. 39031, 3 July 1979). It should be noted that mud logs from the J-series included no record of such additives. A possible source of phenols other than biocide additives is the petroleum products found in the drilling fluids (John Farrington, personal communication).

Toxicity

Four-day LC50 values demonstrate a wide variability in the toxicity of the five drilling fluids tested (Table 4). 96-h LC_{50} values were 73.8 ppm for J-5 and 88.3 ppm for J-7 exposed stage I larvae. In contrast, the 96-h LC_{50} for stage I and stage IV larvae exposed to J-4 drilling fluid was greater than the highest concentration (500 ppm) tested, and there were no significant differences in the percent survival for these stages at concentrations of 10, 50, 100, 250 and 500 ppm. The 96-h LC_{50} for J-6 exposed stage I larvae, 213.4 ppm, was greater than for J-5 and J-7 by over a factor of two. Overall control mortality for these experiments was 33 percent, largely attributable to cannabilism.

Table 3. Physical and chemical characteristics of the J-series and Mobile Bay (5/29/79) drilling fluids.[a]

Drilling Fluid	pH	Density (lbs/gal)	Fe(%)	Al(%)	Ba(%)	Pb (ppm)	Cr (ppm)	Zn (ppm)	H_2O (%)	Diesel (%) Oil	Total Resolved Aliphatics (µg/l)	Total Resolved Aromatics (µg/l)	Phenols (ppm)
J-4	11.0	9.1	3.2	8.0	4.1	40.2	96.4	225	88.3	0	17,000	27,300	<0.25
J-5	11.4	9.3	2.6	6.5	9.3	41.7	201	271	82.6	2	1,320,000	380,000	0.45
J-6	11.0	9.3	2.3	5.9	10.6	41.3	160	250	79.3	3	Not available	555,000	<0.25
J-7	11.5	9.3	1.1	2.5	11.7	18.5	128	175	79.0	4	1,270,000	376,000	0.33
MB-5-29	11.9	9.1	2.7	6.1	8.2	37.8	1810.0	98.6	80.0	0.27[b]	-	-	1.3

[a]Analyses performed at Science Applications, Inc., La Jolla, CA; New England Aquarium, Boston, MA; University of West Florida, Pensacola, FL; and Energy Resources Co., Inc., Cambridge, MA.

[b]No. 2 fuel oil.

Table 4. Four day median lethal concentration (96-h LC_{50}) values (± 95 confidence limits) for drilling fluid exposed lobster larvae.

Stage	Drilling Fluid	96-h LC_{50} (ppm) (± 95% CL)
I	MB-5-29	189.54 (2.03)
III	MB-5-29	117.17 (4.50)
I	J-4	>500
IV	J-4	>500
I	J-5	73.76 (2.43)
I	J-6	213.35 (7.26)
I	J-7	88.29 (2.05)

Results for MB-5-29 exposed stage I larvae were similar to those obtained for J-6, with a 96-h LC_{50} of 189.5 ppm. An estimation of the 96-h LC_{50} could not be determined for stage II larvae with this drilling fluid because of an insufficient number of mortality determinations between 0 and 100 percent. However, decreased survival rates were observed at concentrations as low as 50 ppm and 100 ppm. The 96-h LC_{50} was 117.2 ppm for MB-5-29 exposed stage III larvae. The percent mortality at 96 h for control larvae and larvae tested with a range of drilling fluid exposure concentrations is presented in Fig. 3.

Survival data comparisons for larvae exposed to control seawater, Georges Bank sediment (500 ppm), and barite (500 ppm) show no significant differences (Tables 5 and 6). The experiment evaluating the toxic effects on larvae of ingesting a drilling fluid exposed food source also revealed no significant differences in percent survival between groups (Tables 7 and 8).

Growth Rates and Molting

Significant changes in larval growth rates and developmental times resulted from exposures to most of the drilling fluids tested. Decreases in mean dry weights of stage I larvae were found after a 24-h exposure to J-5, 6, and 7 at the highest concentrations tested, with significant differences at 500 ppm of J-6 ($p < 0.005$) and 500 ppm of J-7 ($p < 0.01$) (Tables 9 and 10). Significant reductions in the dry weights of stage I larvae were also observed with a 96-h exposure to 100 ppm of J-5, 6, and 7. Highly significant ($p < 0.001$) reductions occurred with exposure to 50 ppm of J-5 but exposure to the same concentration of J-6 and 7 resulted in no such reductions. Developmental time of larvae exposed to high concentrations of these drilling fluids was longer than in the control groups, as demonstrated by the percentages of larvae that molted to stage II by 96 h.

Figure 3. Percent mortality at 96 h of control and drilling fluid exposed stage I lobster larvae

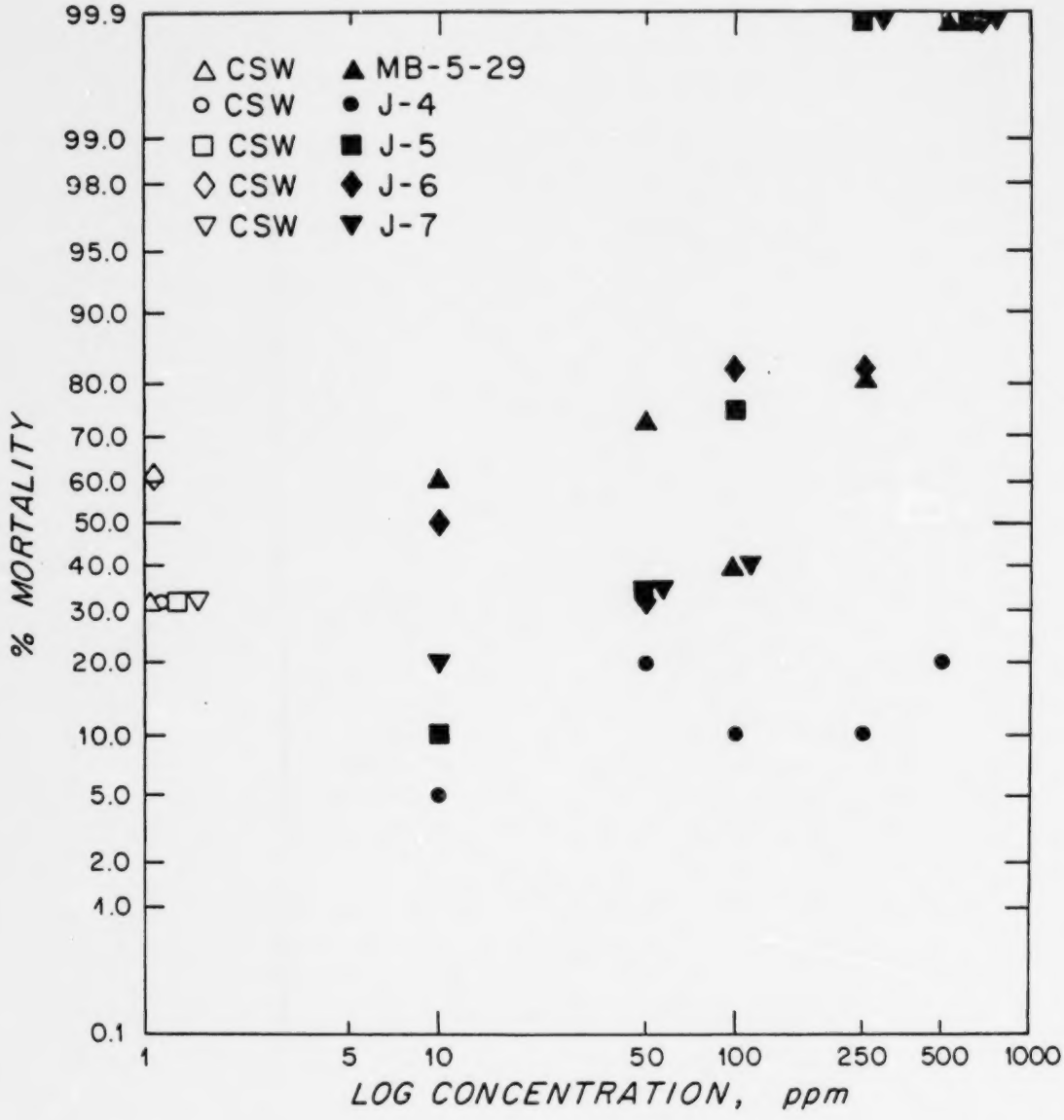

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Table 5. Percent survival at 96 h of control, Georges Bank - 500 ppm and barite - 500 ppm exposed stage I larvae.[a]

Group	% survival $\bar{x}$ ± 1 S.E.
CSW	73.3 ± 8.4
GB-500 ppm	63.3 ± 3.3
Bar-500 ppm	60.0 ± 7.3

[a]Mean value of 4 replicates ± 1 S.E.

Table 6. ANOVA of percent survival comparison of stage I larvae.

Source of variation	df	SS	MS	F-ratio
Among groups	2.00	577.78	288.89	1.07 ns
CSW, GB-500 vs BAR-500	1.00	277.78	277.78	1.02 ns
CSW vs GB-500	1.00	300.00	300.00	1.22 ns
Within groups	15.00	4,066.67	271.11	-

Table 7. Percent survival at 96 h of stage I larvae fed Artemia nauplii cultured in uncontaminated seawater, Georges Bank sediment and J-5 drilling fluid.[a]

Group	% survival $\bar{x}$ ± 1 S.E.
CSW	70.0 ± 12.9
GB-50 ppm	65.0 ± 5.0
J-5-50 ppm	75.0 ± 5.0
J-5-100 ppm	80.0 ± 8.2
J-5-250 ppm	65.0 ± 12.6
J-5-500 ppm	60.0 ± 0.0

[a]Mean value of 4 replicates ± 1 S.E.

Table 8. ANOVA of percent survival comparison of stage I larvae fed Artemia nauplii cultured in uncontaminated seawater, Georges Bank sediment and J-5 drilling fluid.

Source of variation	df	SS	MS	F-ratio
Among groups	5.00	1,083.33	216.67	0.74 ns
CSW and GBART 50 vs J-5 ART- (All concentrations)	1.00	33.33	33.33	0.12 ns
CSW vs GBART 50	1.00	50.00	50.00	0.13 ns
Within groups	18.00	5,300.00	294.44	-

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Table 9. Mean dry weights (mg) of control and drilling fluid exposed stage I larvae at 24 h and 96 h.

Drilling Fluid		Group								
			GB							
	CSW	50	100	250	DM-10	DM-50	DM-100	DM-250	DM-500
J-4 96 h	1.27 (0.03)	-	1.40[f]	1.30 (0.05)	1.22[f]	1.27[f]	1.18[f]	1.26 (0.03)	1.35[a] (0.03)
n=	12		18	15	19	15	18	16	14
% molt to stage II	100		100	95	100	100	100	100	100
J-5 24 h	1.00 (0.05)	1.10 (0.02)	-	-	-	1.12 (0.02)	0.88 (0.10)	-	-
n =	12	8				8	4		
96 h	1.62 (0.05)	1.67 (0.04)	-	-	1.35 (0.10)	1.13[e] (0.04)	0.88[e] (0.02)	-	-
n =	32	16			8	21	2		
% molt to stage II	86	100			75	43	0		
J-6 24 h	1.00 (0.05)	-	-	-	-	-	-	-	0.86[d] (0.03)
n =	13								5
96 h	1.57 (0.08)	-	-	-	1.61 (0.07)	1.43 (0.04)	1.19[b] (0.10)	0.96[e] (0.03)	-
n =	9				14	19	5	5	
% molt to stage II	100				100	95	50	0	

Table 9. (continued)

Drilling Fluid	CSW	GB 50	GB 100	GB 250	Group DM-10	Group DM-50	Group DM-100	Group DM-250	Group DM-500
J-7 24 h	1.00 (0.05)	-	-	-	-	-	-	-	0.86[c] (0.07)
n =	13								5
96 h	1.46 (0.06)	-	-	-	1.26 (0.04)	1.37 (0.05)	1.16[d] (0.03)	-	-
n =	14				16	13	8		
% molt to stage II	100				94	92	75		

[a]P <0.05
[b]P <0.025
[c]P <0.01
[d]P <0.005
[e]P <0.001
[f]Average dry weight/larva from pooled samples.

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Table 10. ANOVA of mean dry weights of control and drilling fluid exposed stage I larvae at 24 h and 96 h.

Drilling Fluid	Source of variation	df	SS	MS	F-ratio
J-4 96 h	Among groups	2.00	0.02	0.01	0.42 ns
	CSW, GB-250 vs DM-250	1.00	0.01	0.01	0.52 ns
	CSW vs GB-250	1.00	0.01	0.01	0.33 ns
	Within groups	38.00	0.82	0.02	-
	Among groups (CSW vs DM-500)	1.00	0.04	0.04	4.36[a]
	Within groups (CSW vs DM-500)	24.00	0.23	0.01	-
J-5 24 h	Among groups	2.00	0.09	0.05	3.26 ns
	CSW, GB-50 vs DM-50	1.00	0.04	0.04	2.80 ns
	CSW vs GB-50	1.00	0.05	0.05	2.71 ns
	Within groups	25.00	0.35	0.01	-
24 h	Among groups (CSW vs DM-100)	1.00	0.04	0.04	1.38 ns
	Within groups (CSW vs DM-100)	14.00	0.41	0.03	-
J-5 96 h	Among groups	2.00	3.88	1.94	38.47[e]
	CSW, GB-50 vs DM-50	1.00	3.85	3.85	77.08[e]
	CSW vs GB-50	1.00	0.02	0.02	0.39 ns
	Within groups	66.00	3.32	0.05	-
	Among groups (CSW vs DM-100)	1.00	1.05	1.05	14.21[e]
	Within groups (CSW vs DM-100)	32.00	2.37	0.07	-
J-6 24 h	Among groups	3.00	0.16	0.05	3.78[b]
	CSW, GB-500, Bar-500 vs DM-500	1.00	0.13	0.13	9.12[d]
	CSW, GB-500 vs Bar-500	1.00	0.00	0.00	0.01 ns
	CSW vs GB-500	1.00	0.03	0.03	1.89 ns
	Within groups	42.00	0.59	0.01	-
J-6 96 h	Among groups (CSW vs DM-100)	1.00	0.46	0.46	9.19[b]
	Within groups (CSW vs DM-100)	12.00	0.60	0.05	-
	Among groups (CSW vs DM-250)	1.00	1.20	1.20	31.25[e]
	Within groups (CSW vs DM-250)	12.00	0.46	0.04	-
J-7 24 h	Among groups	3.00	0.16	0.05	3.50[b]
	CSW, GB-500, Bar-500 vs DM-500	1.00	0.13	0.13	8.22[c]
	CSW, GB-500 vs Bar-500	1.00	0.00	0.00	0.01 ns
	CSW vs GB-500	1.00	0.03	0.03	1.89 ns
	Within groups	42.00	0.65	0.02	-
J-7 96 h	Among groups (CSW vs DM-100)	1.00	0.46	0.46	13.00[d]
	Within groups (CSW vs DM-100)	20.00	0.71	0.04	-

[a] $p < 0.05$
[b] $p < 0.025$
[c] $p < 0.01$
[d] $p < 0.005$
[e] $p < 0.001$

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PAGE NUMBER
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No significant differences in dry weights of stage I larvae resulted from a 96 h exposure to concentrations of J-4 as high as 250 ppm, however, a small increase ($p < 0.05$) resulted with exposure to 500 ppm of J-4. Larvae at all concentrations of J-4 and in the control seawater group had successfully molted to stage II at 96 h. Stage IV larvae also exhibited no significant decrease in dry weight from the controls after a 96 h exposure to 100 ppm and 500 ppm of J-4 drilling fluid (Tables 11 and 12).

Mean dry weights of different larval stages exposed to MB-5-29 (as determined from pooled samples) are listed in Table 13. The three larval stages tested (I, II and III) have decreased 96-h dry weight values at concentrations of 50 ppm and higher as compared to the control seawater values. Stage I larvae exposed to the J-series drilling fluids generally exhibited greater declines in mean dry weights (as compared to controls) than did the MB-5-29 exposed stage I larvae.

There were no significant differences between dry weight values for stage I larvae exposed to either the Georges Bank sediment, barite, or the control seawater even at the highest concentration tested (500 ppm)(Tables 14 and 15). Dry weight comparisons were also made among the groups of stage I lobsters fed with either uncontaminated Artemia nauplii or with Artemia exposed to different concentrations of drilling fluid (Tables 16 and 17). Larvae fed with Artemia which had been contaminated with concentrations of J-5 ranging from 50 to 250 ppm showed no difference in dry weights from the control groups. A significant difference ($p < 0.025$) was found between control lobsters and lobsters fed with J-5-500 ppm contaminated Artemia.

Respiration Rates

Respiration rates of stage I larvae were affected by 24-h and 96-h exposures to concentrations of drilling fluids from the J-series (Tables 18 and 19). Significant reductions in respiration rates (expressed as $\mu l\ O_2$ consumed/h) of J-5 exposed larvae were observed with exposure to 100 ppm at 24 h and 50 ppm at 96 h. J-6 and J-7 exposure at a concentration of 100 ppm also resulted in significantly decreased respiration rates after a 96-h exposure. There was no significant difference in respiration rates of J-4-100 ppm exposed larvae; however, the mean respiration value was low as compared to the control seawater and Georges Bank sediment-100 ppm groups.

Respiration rates calculated on a weight-specific basis (expressed as $\mu l\ O_2$ consumed/hr·mg dry weight^{-1}) were generally not different for the various groups of control and exposed stage I larvae (Tables 20 and 21). After 24 h of exposure, only J-5-100 ppm exposed larvae had significantly reduced weight-specific respiration values ($p < 0.005$); J-6-100 ppm exposed larvae had increased, but not significantly different, weight-specific respiration values. No significant differences were found for these stage I larvae exposed to the J-series for 96 h, although the mean weight-specific respiration value was reduced for each drilling fluid in the J-series as compared to the controls. Significant reductions in the dry weights of drilling fluid exposed larvae were responsible for the observed differences between the absolute respiraton rates and the weight-specific respiration rates of control and experimental groups.

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Table 11. Mean dry weights (mg) of control and J-4 drilling fluid exposed stage IV larvae at 96 h.

Group	Dry weight, mg $\bar{x}$ ± 1 S.E.
CSW	9.90 ± 0.63
J-4-100 ppm	11.43 ± 0.48
J-4-500 ppm	9.75 ± 0.80

Table 12. ANOVA of mean dry weights of control and J-4 drilling fluid exposed stage IV larvae at 96 h.

Source of variation	df	SS	MS	F-ratio
Among groups (CSW vs J-4-100)	1	18.57	18.57	3.67 ns
Within groups (CSW vs J-4-100)	30	151.69	5.06	-
Among groups (CSW vs J-4-500)	1	0.12	0.12	0.02 ns
Within groups (CSW vs J-4-500)	22	131.73	5.99	-

Table 13. Mean dry weights (mg) of control and MB-5-29 drilling fluid exposed lobster larvae at 96 h.[a]

	Group						
Stage	CSW	1 ppm	10 ppm	50 ppm	100 ppm	250 ppm	500 ppm
I	0.96	0.95	0.98	0.74	0.75	0.69	-
II	1.70	1.76	2.12	1.43	1.20	1.03	-
III	2.78	-	-	2.32	2.24	1.92	1.81

[a]From pooled samples.

Table 14. Mean dry weights (mg) of control, Georges Bank - 500 ppm exposed and barite - 500 ppm exposed stage I larvae at 24 h and 96 h.

Group	Dry weight, mg $\bar{x}$ ± 1 S.E. (n)	
	24 h	96 h
CSW	1.00 ± 0.05 (13)	1.45 ± 0.05 (32)
GB-500	1.07 ± 0.02 (14)	1.58 ± 0.04 (30)
Bar-500	1.03 ± 0.03 (14)	1.59 ± 0.05 (33)

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Table 15. ANOVA of mean dry weights of control, Georges Bank - 500 ppm exposed and barite - 500 ppm exposed stage I larvae.

Source of variation	df	SS	MS	F-ratio
24 h				
Among groups	2	0.03	0.02	1.02 ns
CSW, GB-500 vs Bar-500	1	0.00	0.00	0.01 ns
CSW vs Bar-500	1	0.03	0.03	1.89 ns
Within groups	38	0.57	0.01	-
96 h				
Among groups	2	0.36	0.18	2.33 ns
CSW, GB-500 vs Bar-500	1	0.11	0.11	1.38 ns
CSW vs GB-500	1	0.25	0.25	3.81 ns
Within groups	92	7.09	0.08	-

Table 16. Mean dry weights (mg) at 96 h of stage I larvae fed *Artemia* nauplii cultured in uncontaminated seawater, Georges Bank sediment and J-5 drilling fluid.

Group	Dry weight, mg $\bar{x}$ ± 1 S.E.
CSW	1.65 ± 0.09
GB-50 ppm	1.81 ± 0.05
J-5 50 ppm	1.76 ± 0.04
J-5 100 ppm	1.66 ± 0.05
J-5 250 ppm	1.60 ± 0.07
J-5 500 ppm	1.42 ± 0.04

Table 17. ANOVA of mean dry weights at 96 h of stage I larvae fed *Artemia* nauplii cultured in uncontaminated seawater, Georges Bank sediment and J-5 drilling fluid.

Source of variation	df	SS	MS	F-ratio
Among groups	5.00	1.11	0.22	5.57[b]
CSW, Art GB-50 vs ART J-5-50	1.00	0.01	0.01	0.12 ns
CSW vs Art J-5-100	1.00	0.001	0.001	0.01 ns
CSW vs Art J-5-250	1.00	0.01	0.01	0.23 ns
CSW vs Art J-5-500	1.00	0.33	0.33	7.07[a]
CSW vs Art GB-50	1.00	0.13	0.13	2.49 ns
Within groups	70.00	2.78	0.04	-

[a] $p < 0.025$
[b] $p < 0.001$

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Table 18. Mean respiration rates (µl O_2/h) of control and drilling fluid exposed stage I larvae at 24 h and 96 h*.

Drilling Fluid	h	Group: CSW	GB: 50	GB: 100	GB: 500	Bar-500	DM-50	DM-100	DM-500
J-4	96	9.50	–	9.05	–	–	–	8.01	–
24°C		(0.58)	–	(0.45)	–	–	–	(0.61)	–
	n =	7	–	7	–	–	–	5	–
J-5	24	4.69	5.14	–	–	–	5.01	2.23[b]	–
23°C		(0.38)	(0.50)	–	–	–	(0.36)	(0.49)	–
	n =	21	13	–	–	–	14	6	–
24°C	96	7.06	8.11	–	–	–	4.91[c]	–	–
		(0.51)	(0.48)	–	–	–	(0.42)	–	–
	n =	21	14	–	–	–	21	–	–
J-6	24	4.23	–	–	3.64	4.51	–	–	3.81
20°C		(0.45)	–	–	(0.26)	(0.55)	–	–	(0.39)
	n =	7	–	–	7	7	–	–	5
	96	5.73	–	–	–	–	–	3.78[b]	–
20°C		(0.38)	–	–	–	–	–	(0.13)	–
	n =	7	–	–	–	–	–	5	–
J-7	96	4.88	–	–	–	–	–	2.96[a]	–
18°C		(0.33)	–	–	–	–	–	(0.42)	–
	n =	7	–	–	–	–	–	4	–

*Assay temperature and sample size included.

[a] $P < 0.01$

[b] $P < 0.005$

[c] $P < 0.001$

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Table 19. ANOVA of absolute respiration rates of control and drilling fluid exposed stage I larvae at 24 h and 96 h.

Drilling Fluid	h	Source of variation	df	SS	MS	F-ratio
J-4	96	Among groups	2.00	6.50	3.25	1.47 ns
		CSW, GB-100 vs DM-100	1.00	5.24	5.24	2.42 ns
		CSW vs GB-100	1.00	1.26	1.26	0.54 ns
		Within groups	16.00	35.47	2.22	-
J-5	24	Among groups (CSW vs DM-100)	1.00	28.34	28.34	10.23[b]
		Within groups (CSW vs DM-100)	25.00	69.28	2.77	-
J-5	24	Among groups	2.00	1.84	0.92	0.33 ns
		CSW, GB-50 vs DM-50	1.00	0.20	0.20	0.07 ns
		CSW vs GB-50	1.00	1.64	1.64	0.52 ns
		Within groups	45.00	124.45	2.77	-
J-5	96	Among groups	2.00	96.04	48.02	11.27[b]
		CSW, GB-50 vs DM-50	1.00	88.30	88.30	20.65[c]
		CSW vs GB-50	1.00	9.29	9.29	2.02 ns
		Within groups	53.00	225.78	4.26	-
J-6	24	Among groups	3.00	3.13	1.04	0.84 ns
		CSW, GB-500, Bar-500 vs DM-500	1.00	0.40	0.40	0.32 ns
		CSW, GB-500 vs Bar-500	1.00	1.54	1.54	1.15 ns
		CSW vs GB-500	1.00	1.19	1.19	1.25 ns
		Within groups	22.00	27.23	1.24	-
J-6	96	Among groups (CSW vs DM-100)	1.00	9.68	9.68	14.21[b]
		Within groups (CSW vs DM-100)	10.00	6.13	0.68	-
J-7	96	Among groups (CSW vs DM-100)	1.00	9.36	9.36	12.46[a]
		Within groups (CSW vs DM-100)	9.00	6.76	0.75	-

[a] $P < 0.01$
[b] $P < 0.005$
[c] $P < 0.001$

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Table 20. Mean weight-specific respiration rates (µl O_2/h/mg dry wt.) of control and drilling fluid exposed stage I larvae at 24 h and 96 h*.

Drilling Fluid	h	Group: GB CSW	GB 50	GB 100	500	Bar-500	DM-50	DM-100	DM-500
J-4	96	5.50	-	5.09	-	-	-	4.76	-
24°C		(0.14)	-	(0.18)	-	-	-	(0.27)	-
	n =	7	-	7	-	-	-	5	-
J-5	24	4.61	4.70	-	-	-	4.48	2.51[a]	-
23°C		(0.31)	(0.48)	-	-	-	(0.36)	(0.55)	-
	n =	21	13	-	-	-	14	6	-
	96	4.67	4.73	-	-	-	4.31	-	-
24°C		(0.24)	(0.23)	-	-	-	(0.35)	-	-
	n =	21	14	-	-	-	21	-	-
J-6	24	3.90	-	-	3.36	4.04	-	-	4.43
20°C		(0.41)	-	-	(0.24)	(0.50)	-	-	(0.45)
	n =	7	-	-	7	7	-	-	5
	96	3.83	-	-	-	-	-	3.29	-
20°C		(0.12)	-	-	-	-	-	(0.28)	-
	n =	7	-	-	-	-	-	5	-
J-7	96	3.34	-	-	-	-	-	2.55	-
18°C		(0.23)	-	-	-	-	-	(0.36)	-
	n =	7	-	-	-	-	-	4	-

*Assay temperature and sample size included.

[a] $P < 0.005$

Table 21. ANOVA of weight-specific respiration rates of control and drilling fluid exposed stage I larvae at 24 h and 96 h.

Drilling Fluid	h	Source of variation	df	SS	MS	F-ratio
J-4	96	Among groups	2.00	1.61	0.81	3.50 ns
		CSW, GB-100 vs DM-100	1.00	1.04	1.04	4.14 ns
		CSW vs GB 100	1.00	0.58	0.58	3.08 ns
		Within groups	16.00	3.69	0.23	-
J-5	24	Among groups (CSW vs DM-100)	1.00	20.53	20.53	10.24[a]
		Within groups (CSW vs DM-100)	25.00	50.13	2.01	-
	24	Among groups	2.00	0.33	0.16	0.07 ns
		CSW, GB-50 vs DM-50	1.00	0.27	0.27	0.12 ns
		CSW vs GB-50	1.00	0.38	0.38	0.17 ns
		Within groups	45.00	99.87	2.22	-
	96	Among groups	2.00	1.97	0.99	0.61 ns
		CSW, GB-50 vs DM-50	1.00	1.95	1.95	1.24 ns
		CSW vs GB-50	1.00	0.02	0.02	0.02 ns
		Within groups	53.00	85.30	1.61	-
J-6	24	Among groups	3.00	3.52	1.17	1.08 ns
		CSW, GB-500, Bar 500 vs DM 500	1.00	1.76	1.76	1.64 ns
		CSW, GB 500 vs Bar 500	1.00	0.77	0.77	0.70 ns
		CSW vs GB 500	1.00	1.60	1.60	1.37 ns
		Within groups	22.00	23.99	1.09	-
	96	Among groups (CSW vs DM 100)	1.00	0.85	0.85	3.87 ns
		Within groups (CSW vs DM 100)	10.00	2.21	0.22	-
J-7	96	Among groups (CSW vs DM 100)	1.00	1.58	1.58	3.79 ns
		Within groups (CSW vs DM 100)	9.00	3.74	0.42	-

[a] $p < 0.005$

Respiration rates (absolute and weight-specific) were determined for the 24 h, 48 h, 72 h, and 96 h exposure periods with J-4 drilling fluid of stage IV larvae (Tables 22-25). There was no significant difference between control seawater larvae, and 100 ppm and 500 ppm exposed larvae. Both control and drilling fluid exposed larvae had decreased weight-specific respiration rates at 96 h. By this time 85 percent of the larvae from both groups had molted to stage V, and as shown by Capuzzo and Lancaster (1979), there is a decline in the respiration rate following the molt to the first post-larval stage.

Table 22. Mean respiration rates (µl O_2/h) of control and J-4 drilling fluid exposed stage IV larvae.

Group	x µl O_2/h (± 1 S.E.) 24 h	48 h	72 h	96 h
CSW	20.12 (1.16)	22.42 (1.92)	19.13 (0.95)	22.08 (1.05)
n =	21	7	21	7
DM-100	20.56 (1.12)	20.82 (0.98)	19.30 (1.18)	17.69 (1.12)[a]
n =	21	7	21	7
DM-500	18.16 (1.86)	-	17.57 (1.16)	-
n =	7	-	7	-

[a] p 0.025

Table 23. ANOVA of absolute respiration rates of control and J-4 drilling fluid exposed stage IV lobster larvae.

Source of variation		df	SS	MS	F-ratio
24 h	Among groups (CSW vs DM-100)	1.00	1.42	1.42	0.05 ns
	Within groups (CSW vs DM-100)	40.00	1094.80	26.70	-
	Among groups (CSW vs DM-500)	1.00	20.07	20.07	0.74 ns
	Within groups (CSW vs DM-500)	26.00	708.98	27.27	-
48 h	Among groups (CSW vs DM-100)	1.00	8.91	8.91	0.55 ns
	Within groups (CSW vs DM-100)	12.00	194.03	16.17	-
72 h	Among groups (CSW vs DM-100)	1.00	0.30	0.30	0.01 ns
	Within groups (CSW vs DM-100)	40.00	957.63	23.94	-
	Among groups (CSW vs DM-500)	1.00	12.82	12.82	0.77 ns
	Within groups (CSW vs DM-500)	26.00	431.90	16.61	-
96 h	Among groups (CSW vs DM-100)	1.00	67.76	67.76	8.26[a]
	Within groups (CSW vs DM-100)	12.00	98.48	8.21	-

[a] p <0.025

Table 24. Mean weight-specific respiration rates (µl O_2/h/mg dry wt.) of control and J-4 drilling fluid exposed stage IV lobster larvae.

Group	x µl O_2/h/mg dry wt. (± 1 S.E.) 24 h	48 h	72 h	96 h
CSW	2.58 (0.15)	2.58 (0.22)	2.13 (0.09)	2.13 (0.07)
n =	21	7	21	7
DM-100	2.41 (0.09)	2.35 (0.06)	2.03 (0.08)	1.96 (0.10)
n =	21	7	21	7
DM-500	2.06 (0.13)	-	2.37 (0.19)	-
	7	-	7	-

Table 25. ANOVA of weight-specific respiration rates of control and J-4 drilling fluid exposed stage IV larvae.

Source of variation		df	SS	MS	F-ratio
24 h	Among groups (CSW vs DM-100)	1.00	0.32	0.32	1.02 ns
	Within groups (CSW vs DM-100)	40.00	12.66	0.32	-
	Among groups (CSW vs DM-500)	1.00	1.42	1.42	3.67 ns
	Within groups (CSW vs DM-500)	26.00	10.03	0.39	-
48 h	Among groups (CSW vs DM-100)	1.00	0.18	0.18	1.01 ns
	Within groups (CSW vs DM-100)	12.00	2.17	0.18	-
72 h	Among groups (CSW vs DM-100)	1.00	0.09	0.09	0.62 ns
	Within groups (CSW vs DM-100)	40.00	5.95	0.15	-
	Among groups (CSW vs DM-500)	1.00	0.32	0.32	1.66 ns
	Within groups (CSW vs DM-500)	26.00	5.01	0.19	-
96 h	Among groups (CSW vs DM-100)	1.00	0.10	0.10	1.82 ns
	Within groups (CSW vs DM-100)	12.00	0.65	0.05	-

In exposures conducted with 10 ppm MB-5-29 drilling fluid, stage I larvae exhibited significant differences in absolute value and weight-specific respiration rates ($p < 0.05$) at 24 h and 96 h (Tables 26-29). Respiration rates measured for 1 ppm exposed larvae were not different from the control seawater larvae.

Respiration rates (absolute and weight-specific) for stage I larvae exposed to 50, 100, or 500 ppm of Georges Bank sediment or to 500 ppm barite (Tables 30-33) were not significantly different from the control seawater values at both 24 h and 96 h. Lobsters fed with Artemia exposed to 50 ppm drilling fluid (J-5) had respiration rates similar to the control lobsters (Tables 34-37).

Feeding Rates

The results of feeding experiments with drilling fluid exposed and control stage I larvae are presented in Tables 38 and 39. After 24 h, the larvae exposed to J-5-50 ppm consumed an average of 15.5 Artemia nauplii, while the control seawater group and the Georges Bank sediment exposed group consumed an average of 27.3 and 32.8, respectively. A statistical comparison demonstrates a highly significant difference ($p < 0.001$) between control and drilling fluid groups, but no significant difference between the control seawater and Georges Bank sediment groups.

Data from the feeding experiments were converted from the number of Artemia consumed to calories using average dry weight and caloric content for Artemia nauplii. Growth rates of larvae (Table 9) were also converted to hourly caloric values using the caloric equivalents of dry weights of stage I larvae given by Logan and Epifanio (1978). The number of calories available and utilized (Q_G) for growth are considerably lower for the

Table 26. Mean respiration rates (µl O_2/h) of control and MB-5-29 drilling fluid exposed stage I larvae.[a]

Group	x µl O_2/h (± 1 S.E.) 24 h	96 h
CSW	6.69 (0.69)	6.53 (0.57)
DM-1 ppm	5.11 (0.49)	5.82 (0.78)
DM-10 ppm	4.44 (0.34)[c]	4.78 (0.46)[b]

[a]Each value is the mean of seven determinations (± 1 S.E.)
[b]$P < 0.05$
[c]$P < 0.025$

Table 27. ANOVA of absolute respiration rates of control and MB-5-29 drilling fluid exposed stage I larvae.

Source of variation		df	SS	MS	F-ratio
24 h	Among groups (CSW vs DM-1)	1.00	8.73	8.73	3.46 ns
	Within groups (CSW vs DM-1)	12.00	30.31	2.53	-
	Among groups (CSW vs DM-10)	1.00	17.63	17.63	8.48[b]
	Within groups (CSW vs DM-10)	12.00	24.94	2.08	-
96 h	Among groups (CSW vs DM-1)	1.00	1.78	1.78	0.54 ns
	Within groups (CSW vs DM-1)	12.00	39.40	3.28	-
	Among groups (CSW vs DM-10)	1.00	10.79	10.79	5.80[a]
	Within groups (CSW vs DM-10)	12.00	22.33	1.86	

[a]$P < 0.05$
[b]$P < 0.025$

Table 28. Mean weight-specific respiration rates (µl O_2/h/mg dry wt.) of control and MB-5-29 drilling fluid exposed stage I larvae.[a]

Group	x µl O_2/h/mg dry wt. (± 1 S.E.) 24 h	96 h
CSW	3.93 (0.41)	2.51 (0.22)
DM-1 ppm	3.00 (0.29)	2.24 (0.30)
DM-10 ppm	2.61 (0.20)[b]	1.84 (0.18)[b]

[a]Each value is the mean of seven determinations (± 1 S.E.)
[b]$P < 0.05$

Table 29. ANOVA of weight-specific respiration rates of control and MB-5-29 drilling fluid exposed stage I larvae.[a]

Source of variation		df	SS	MS	F-ratio
24 h	Among groups (CSW vs DM-1)	1.00	3.02	3.02	3.46 ns
	Within groups (CSW vs DM-1)	12.00	10.49	0.87	-
	Among groups (CSW vs DM-10)	1.00	6.10	6.10	8.48[a]
	Within groups (CSW vs DM-10)	12.00	8.63	0.72	-
96 h	Among groups (CSW vs DM-1)	1.00	0.001	0.001	0.005 ns
	Within groups (CSW vs DM-1)	12.00	3.12	0.28	-
	Among groups (CSW vs DM-10)	1.00	1.60	1.60	5.80[a]
	Within groups (CSW vs DM-10)	12.00	3.30	0.28	-

[a] $p < 0.05$

Table 30. Mean respiration rates (µl O_2/h) at 24 h and 96 h of control, Georges Bank - 500 ppm exposed and barite - 500 ppm exposed stage I larvae.

Group	x µl O_2/h (± 1 S.E.) 24 h	96 h
CSW	4.07 (0.28) n = 14	5.42 (0.43) n = 14
GB-500	3.89 (0.19) n = 14	5.96 (0.37) n = 13
Bar-500	4.51 (0.55) n = 7	4.88 (0.39) n = 12

Table 31. ANOVA of absolute respiration rates of control, Georges Bank - 500 ppm exposed and barite - 500 ppm exposed stage I larvae.

Source of variation		df	SS	MS	F-ratio
24 h	Among groups	2.00	1.82	0.91	0.88 ns
	CSW, GB-500 vs Bar-500	1.00	1.58	1.58	1.56 ns
	CSW vs GB-500	1.00	0.24	0.24	0.31 ns
	Within groups	32.00	33.15	1.04	-
96 h	Among groups	2.00	7.37	3.69	1.76 ns
	CSW, GB-500 vs Bar-500	1.00	5.36	5.36	2.55 ns
	CSW vs GB-500	1.00	2.02	2.02	0.91 ns
	Within groups	36.00	75.47	2.10	-

Table 32. Mean weight-specific respiration rates (µl O_2/h/mg dry wt.) at 24 h and 96 h of control, Georges Bank - 500 ppm exposed and barite - 500 ppm exposed stage I larvae.

Group	x µl O_2/h/mg dry wt. (± 1 S.E.) 24 h	96 h
CSW	3.70 (0.25) n = 14	3.89 (0.25) n = 14
GB-500	3.65 (0.18) n = 14	3.51 (0.14) n = 13
Bar-500	4.04 (0.49) n = 7	3.29 (0.17) n = 12

Table 33. ANOVA of weight-specific respiration rates of control, Georges Bank - 500 ppm exposed and barite - 500 ppm exposed stage I larvae.

Source of variation		df	SS	MS	F-ratio
24 h	Among groups	2.00	0.76	0.38	0.44 ns
	CSW, GB-500 vs Bar-500	1.00	0.73	0.73	0.87 ns
	CSW vs GB-500	1.00	0.02	0.02	0.02 ns
	Within groups	32.00	27.69	0.87	-
96 h	Among groups	2.00	2.38	1.19	2.39 ns
	CSW, GB-500 vs Bar-500	1.00	1.43	1.43	2.80 ns
	CSW vs GB-500	1.00	0.95	0.95	1.70 ns
	Within groups	36.00	17.92	0.49	-

Table 34. Mean respiration rates (µl O_2/h) at 96 h of stage I larvae fed *Artemia* nauplii cultured in uncontaminated seawater, Georges Bank sediment and J-5 drilling fluid.

Group	x µl O_2/h (+ 1 S.E.)
CSW	8.25 (0.49) n = 21
GB-Art-50	8.83 (0.37) n = 6
J-5-Art-50	8.72 (0.64) n = 7

Table 35. ANOVA of absolute respiration rates of stage I larvae fed Artemia nauplii cultured in uncontaminated seawater, Georges Bank sediment and J-5 drilling fluid.

Source of variation	df	SS	MS	F-ratio
Among groups	2.00	2.28	1.14	0.29 ns
CSW and GB Art 50 vs J-5-Art-50	1.00	0.67	0.67	0.17 ns
CSW vs GB Art-50	1.00	1.61	1.61	0.38 ns
Within groups	31.00	123.32	3.98	-

Table 36. Mean weight-specific respiration rates (µl O_2/h/mg dry wt.) at 96 h of stage I larvae fed Artemia nauplii cultured in uncontaminated seawater, Georges Bank sediment and J-5 drilling fluid.

Group	x µl O_2/h/mg dry wt. (+ 1 S.E.)
CSW	5.02 (0.23) n = 21
GB-Art-50	4.89 (0.21) n = 6
J-5-Art-50	4.90 (0.20) n = 7

Table 37. ANOVA of weight-specific respiration rates of stage I larvae fed Artemia nauplii cultured in uncontaminated seawater, Georges Bank sediment and J-5 drilling fluid.

Source of variation	df	SS	MS	F-ratio
Among groups	2.00	0.14	0.07	0.08 ns
CSW and GB Art 50 vs J-5-Art-50	1.00	0.05	0.05	0.06 ns
CSW vs GB Art-50	1.00	0.09	0.09	0.09 ns
Within groups	31.00	26.01	0.84	-

drilling fluid exposed larvae in comparison with the controls, resulting in reduced growth efficiencies (K_1 Table 40). The growth efficiencies for control larvae are consistent with values reported by Logan and Epifanio (1978) for stage I larvae.

Table 38. Feeding rates of stage I larvae after 24 h exposure to 50 ppm J-5 drilling fluid.[a]

Group	Feeding rate No. Artemia nauplii consumed $larva^{-1} \cdot hr^{-1}$
CSW	27.3 ± 2.8
GB-50 ppm	32.8 ± 2.9
J-5-50 ppm	15.5 ± 2.7

[a] Mean value of 16 determinations ± 1 S.E.

Table 39. ANOVA of feeding rates of stage I larvae after 24 h exposure to 50 ppm J-5 drilling fluid.

Source of variation	df	SS	MS	F-ratio
Among groups	2	2519.29	1259.65	10.24[a]
CSW, GB-50 vs J-5-50	1	2271.76	2271.76	18.07[a]
CSW vs GB-50	1	247.53	247.53	1.93 ns
Within groups	45	5535.19	123.00	-

[a] $p < 0.01$

Table 40. Growth efficiencies (K_1) for control and drilling fluid exposed stage I larvae at 24 h.

Group	Q_C(cal.)[a]	Q_G[b]	K_1[c]
CSW	0.1779	0.035	19.6
GB-50 ppm	0.2141	0.032	14.6
J-5-50 ppm	0.1008	0.005	5.0

[a] based on x values of 16 determinations of number Artemia nauplii consumed/h converted to calories of Artemia per wt.

[b] daily increases in dry weight converted to calories

[c] $K_1 = Q_G/Q_C$

Ammonia Excretion Rates and O:N Ratios

Ammonia excretion rates of stage I larval lobsters were significantly increased with a 96-h exposure to J-5 and J-7 drilling fluids. Values for J-6 exposed larvae were also greater than the control values, although not significantly (Tables 41 and 42). Exposure to J-4 drilling fluid resulted in no differences between exposed and control larvae for both stage I and IV, with the exception of a significant increase in the 72-h ammonia excretion rates of stage IV larvae exposed to 500 ppm-J-4 (Tables 43 and 44).

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Table 41. Mean ammonia excretion rates and O:N ratios (± 1 S.E.) of controls and drilling fluid exposed stage I larvae at 24 h and 96 h.

Drilling Fluid	h	Group CSW	GB-50	DM-50	DM-100
J-4	96	0.28	-	-	0.27
µg NH_4 -N/h/mg dry wt		(±0.05)	-	-	(0.03)
		26.84	-	-	22.61
O:N		(±3.94)	-	-	(1.12)
n =		3	-	-	5
J-5	24	0.19	-	-	0.18
µg NH_4 -N/h/mg dry wt		(±0.03)	-	-	(±0.04)
		24.87	-	-	21.05
O:N		(±1.09)	-	-	(±5.06)
n =		3	-	-	5
J-5	96	0.37	0.33	0.47[b]	-
µg NH_4 -N/h/mg dry wt		(±0.06)	(±0.02)	(±0.03)	-
		18.47	17.26	13.73[b]	-
O:N		(±1.64)	(±1.71)	(±1.18)	-
n =		13	10	14	-
J-6	96	0.34	-	-	0.44
µg NH_4 -N/h/mg dry wt		(±0.03)	-	-	(±0.07)
		15.50	-	-	10.42[a]
O:N		(±1.20)	-	-	(±1.94)
n =		14	-	-	5
J-7	96	0.14	-	-	0.35[a]
µg NH_4 -N/h/mg dry wt		(±0.02)	-	-	(±0.07)
		22.75	-	-	12.73[a]
O:N		(±1.48)	-	-	(±1.96)
n =		6	-	-	6

[a] $p < 0.05$
[b] $p < 0.025$

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Table 42. ANOVA of ammonia excretion rates and O:N ratios of control and drilling fluid exposed stage I larvae at 24 h and 96 h.

Drilling Fluid	Source of variation	df	SS	MS	F-ratio
J-4 96 h µg NH_4^+-N/h/mg dry wt.	Among groups (CSW vs DM-100)	1.00	0.0003	0.0003	0.0653 ns
	Within groups (CSW vs DM-100)	6.00	0.0305	0.0050	-
J-4 96 h O:N ratio	Among groups (CSW vs DM-100)	1.00	33.51	33.51	1.70 ns
	Within groups (CSW vs DM-100)	6.00	118.47	19.74	-
J-5 24 h µg NH_4^+-N/h/mg dry wt.	Among groups (CWS vs DM-100)	1.00	0.0001	0.0001	0.0286 ns
	Within groups (CSW vs DM-100)	7.00	0.0425	0.0060	-
J-5 96 h µg NH_4^+-N/h/mg dry wt.	Among groups	2.00	0.13	0.06	2.98 ns
	CSW, GB-50 vs DM-50	1.00	0.12	0.12	5.72[b]
	CSW vs GB-50	1.00	0.01	0.01	0.28 ns
	Within groups	34.00	0.74	0.02	-
J-5 24 h O:N ratio	Among groups (CSW vs DM-100)	1.00	27.37	27.37	0.32 ns
	Within groups (CSW vs DM-100)	6.00	519.22	86.54	-
J-5 96 h O:N ratio	Among groups	2.00	162.89	81.44	2.96 ns
	CSW, GB-50 vs DM-50	1.00	154.59	154.59	5.74[b]
	CSW vs GB-50	1.00	8.29	8.29	0.26 ns
	Within groups	34.00	934.42	27.48	-
J-6 96 h µg NH_4^+-N/h/mg dry wt.	Among groups (CSW vs DM-100)	1.00	0.04	0.04	2.13 ns
	Within groups (CSW vs DM-100)	17.00	0.28	0.02	-
J-6 96 h O:N ratio	Among groups (CSW vs DM-100)	1.00	94.99	94.99	4.79[a]
	Within groups (CSW vs DM-100)	17.00	337.17	19.83	-

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Table 42. (continued)

Drilling Fluid	Source of variation	df	SS	MS	F-ratio
J-7 96 h µg NH_4^+-N/h/mg dry wt.	Among groups (CSW vs DM-100)	1.00	0.11	0.11	6.14[a]
	Within groups (CSW vs DM-100)	8.00	0.14	0.02	-
J-7 96 h O:N ratio	Among groups (CSW vs DM-100)	1.00	150.59	150.59	7.54[a]
	Within groups (CSW vs DM-100)	6.00	119.80	19.97	-

[a] $p < 0.05$
[b] $p < 0.025$

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Table 43. Mean ammonia excretion rates of control and J-4 drilling fluid exposed stage IV larvae.

Hour of exposure	x µg NH_4^+-N/h/mg dry wt. (± 1 S.E.) 24 h	48 h	72 h	96 h
CSW	0.21 (0.03)	0.30 (0.05)	0.17 (0.02)	0.19 (0.06)
n =	7	7	13	4
DM-100	0.16 (0.02)	0.24 (0.04)	0.16 (0.02)	0.16 (0.03)
n =	6	7	13	4
DM-500	0.20 (0.03)	-	0.31 (0.02)[a]	-
n =	5	-	4	-

Table 44. ANOVA of ammonia excretion rates of control and J-4 drilling fluid exposed stage IV larvae.

Source of variation		df	SS	MS	F-ratio
24 h	Among groups (CSW vs DM-100)	1.00	0.004	0.004	0.67 ns
	Within groups (CSW vs DM-100)	11.00	0.06	0.005	-
	Among groups (CSW vs DM-500)	1.00	0.00	0.00	0.01 ns
	Within groups (CSW vs DM-500)	10.00	0.08	0.008	-
48 h	Among groups (CSW vs DM-100)	1.00	0.01	0.01	0.78 ns
	Within groups (CSW vs DM-100)	12.00	0.17	0.01	-
72 h	Among groups (CSW vs DM-100)	1.00	0.001	0.001	0.08 ns
	Within groups (CSW vs DM-100)	24.00	0.15	0.006	-
	Among groups (CSW vs DM-500)	1.00	0.06	0.06	9.33[a]
	Within groups (CSW vs DM-500)	15.00	0.10	0.01	-
96 h	Among groups (CSW vs DM-100)	1.00	0.003	0.003	0.33 ns
	Within groups (CSW vs DM-100)	6.00	0.05	0.01	-

[a] $p < 0.01$

Values for the atomic ratio of oxygen consumed and ammonia-N excreted are given in Tables 41 and 42. For J-5, 6, and 7 exposed stage I larvae, O:N values were significantly lower after the four-day exposure; exposure to J-4 resulted in no such difference (Figure 4). The average O:N ratio of the control larvae was 21.7 and is within the range previously reported by Capuzzo and Lancaster (1979).

O:N ratios were determined daily for stage IV larvae during the 96-h exposure to a range of concentrations of J-4. No differences were shown, except for a large decrease at 72 h with a 500 ppm exposure (Table 45 and 46).

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Figure 4. Mean O:N ratios (± 1 S. E.) at 96 h of control and J-series drilling fluid exposed stage I lobster larvae

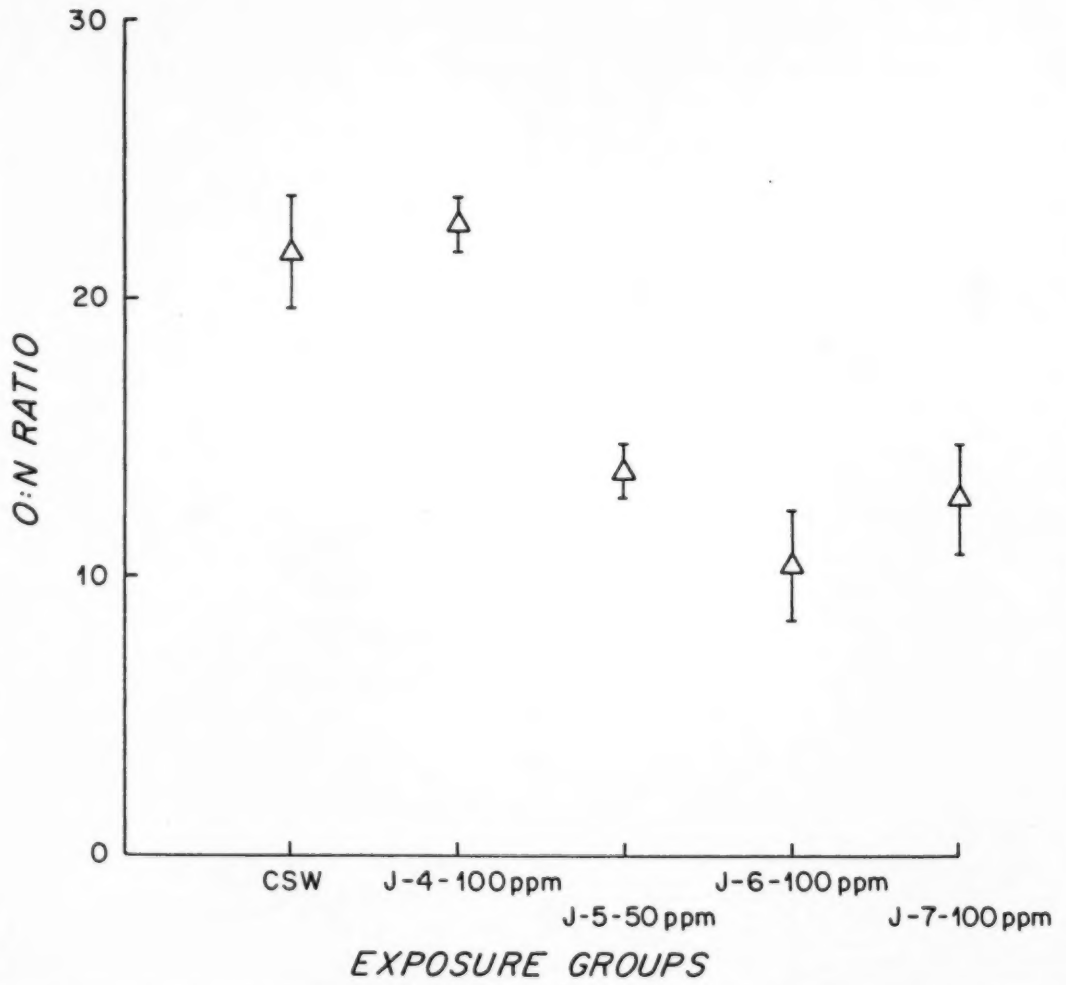

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Table 45. Mean O:N ratios of control and J-4 drilling fluid exposed stage IV larvae.

Hour of exposure	O:N ratio (± 1 S.E.) 24 h	48 h	72 h	96 h
CSW	17.52 (3.05)	11.62 (1.00)	18.47 (2.35)	18.11 (4.83)
n =	7	7	13	4
DM-100	18.15 (1.36)	14.92 (2.82)	18.44 (2.83)	16.94 (2.28)
n =	7	7	13	4
DM-500	14.50 (2.37)	-	9.31 (1.10)	-
n =	5	-	4	-

Table 46. ANOVA of O:N ratios of control and J-4 drilling fluid exposed stage IV larvae.

Source of variation		df	SS	MS	F-ratio
24 h	Among groups (CSW vs DM-100)	1.00	236.63	236.63	0.99 ns
	Within groups (CSW vs DM-100)	12.00	2865.70	238.81	-
	Among groups (CSW vs DM-500)	1.00	26.51	26.51	0.53 ns
	Within groups (CSW vs DM-500)	10.00	503.11	50.31	-
48 h	Among groups (CSW vs DM-100)	1.00	38.15	38.15	1.21 ns
	Within groups (CSW vs DM-100)	12.00	377.04	31.42	-
72 h	Among groups (CSW vs DM-100)	1.00	0.003	0.003	0.00 ns
	Within groups (CSW vs DM-100)	24.00	1924.61	83.68	-
	Among groups (CSW vs DM-500)	1.00	256.43	256.43	4.38 ns
	Within groups (CSW vs DM-500)	15.00	879.15	58.61	-
96 h	Among groups (CSW vs DM-100)	1.00	2.76	2.76	0.05 ns
	Within groups (CSW vs DM-100)	6.00	342.38	57.06	-

Comparisons of ammonia excretion rates and O:N values for Georges Bank - 500 ppm, barite - 500 ppm and control seawater groups demonstrate no significant differences (Tables 47 and 48). The feeding experiments also showed no significant differences between O:N ratios of the larvae fed either drilling fluid contaminated Artemia, Georges Bank sediment exposed Artemia, and uncontaminated Artemia, although there was a trend toward reduction of O:N values in the lobsters fed either Georges Bank sediment exposed Artemia or drilling fluid exposed Artemia (Tables 49 and 50).

Biochemical composition

Biochemical compositions of stage I and IV larval lobsters are presented in Tables 51 and 52. Significant increases ($p < 0.01$) in protein content were found in stage I larvae exposed to 50 ppm J-5, which is below the 96-h LC_{50}. Earlier studies by Capuzzo and Lancaster (1981) on oil-exposed

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Table 47. Mean ammonia excretion rates and O:N ratios (± 1 S.E.) of control, Georges Bank - 500 ppm exposed, and barite - 500 ppm exposed stage I larvae at 96 h.

Group	$\bar{x}$ (± 1 S.E.) $\bar{x}$ µg NH_4^+-N/h/mg dry wt.	O:N
CSW n = 19	0.28 (0.03)	24.26 (3.91)
GB-500 n = 13	0.31 (0.14)	17.35 (3.26)
Bar-500 n = 9	0.22 (0.04)	19.46 (4.17)

Table 48. ANOVA of ammonia excretion rates and O:N ratios of control, Georges Bank exposed and barite exposed stage I larvae.

Source of variation	df	SS	MS	F-ratio
µg NH_4^+-N/h/mg dry wt.				
Among groups	2.00	0.05	0.02	1.15 ns
CSW, GB-500 vs Bar-500	1.00	0.04	0.04	2.04 ns
CSW vs GB-500	1.00	0.01	0.01	0.26 ns
Within groups	38.00	0.75	0.02	-
O:N Ratio				
Among groups	2.00	390.88	195.44	0.92 ns
CSW, GB-500 vs Bar-500	1.00	28.40	28.40	0.13 ns
CSW vs GB-500	1.00	362.47	362.47	1.58 ns
Within groups	38.00	8115.09	213.56	-

Table 49. Mean ammonia excretion rates and O:N ratios (± 1 S.E.) at 96 h of stage I larvae fed *Artemia* nauplii cultured in uncontaminated seawater, Georges Bank sediment and J-5 drilling fluid.

Group	$\bar{x}$ (± 1 S.E.) $\bar{x}$ µg NH_4^+-N/h/mg dry wt.	O:N
CSW	0.35 (0.05) n = 16	20.04 (1.69) n = 16
GB-Art-50	0.39 (0.04) n = 7	14.90 (1.27) n = 6
J-5-Art-50	0.46 (0.06) n = 7	14.27 (1.77) n = 7

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Table 50. ANOVA of ammonia excretion rates and O:N ratios of stage I larvae fed Artemia nauplii cultured in uncontaminated seawater, Georges Bank sediment and J-5 drilling fluid.

Source of variation	df	SS	MS	F-ratio
µg NH_4^+-N/h/mg dry wt.				
Among groups	2.00	0.07	0.03	1.19 ns
CSW and GB Art-50 vs J-5-Art-50	1.00	0.06	0.06	2.23 ns
CSW vs GB Art-50	1.00	0.01	0.01	0.19 ns
Within groups	27.00	0.75	0.03	-
O:N Ratio				
Among groups	2.00	216.46	108.23	3.26 ns
CSW and GB Art-50 vs J-5-Art-50	1.00	115.01	115.01	3.14 ns
CSW vs GB Art-50	1.00	101.45	101.45	2.80 ns
Within groups	26.00	862.93	33.19	-

larval lobsters have shown similar results. Protein:lipid ratios were also significantly greater ($p < 0.05$) in exposed stage I larvae, whereas no significant differences were observed among control and exposed larvae for percent ash, carbohydrate, and lipid values.

Biochemical determinations were made on stage IV larvae after a 96-h exposure to 100 ppm J-4 drilling fluid. A significant increase ($p < 0.05$) in lipid content was observed in the exposed animals. Protein, carbohydrate, and ash contents of the stage IV larvae were not different from the controls. Both control and exposed stage IV larvae had higher ash, carbohydrate, and lipid contents than stage I larvae.

Barium and calcium composition

Barium and calcium concentrations for control, barite-500 ppm exposed and J-4-250 ppm and 500 ppm exposed stage I larvae are given in Table 53. A barium concentration of 1.43 µg/mg dried tissue was found for the barite-exposed larvae; barium was not detected in the other groups. The 250 ppm and 500 ppm drilling fluid exposed larvae had the highest calcium concentrations, 43.8 and 33.0 µg/mg, respectively. Control larvae values were 29.2 µg/mg Ca and barite-exposed values were 24.4 µg/mg Ca.

Table 51. Biochemical analyses of control and drilling fluid exposed lobster larvae.

Component Stage Group	% Ash[a]	% Protein[b]	% Carbohydrate[b]	% Lipid[b]	Protein: Lipid[b]
I Control	21.5 ± 1.7	57.8 ± 1.4	1.4 ± 0.2	11.6 ± 0.9	5.0
GB - 50 ppm	23.6 ± 1.6	60.2 ± 0.7	1.2 ± 0.2	11.9 ± 0.3	5.1
J-5-50 ppm	22.8 ± 2.2	63.7 ± 1.2[c]	1.2 ± 0.1	11.4 ± 0.3	5.6
IV Control	31.2 ± 0.9	37.4 ± 1.3	3.5 ± 0.2	17.1 ± 0.2	2.2
J-4-100 ppm	31.6 ± 0.2	35.6 ± 1.4	3.2 ± 0.1	18.3 ± 0.2[c]	1.9

[a]% dry weight basis; mean values of a sample size of 5 with 3 replicates/sample ± 1 S.E.

[b]% ash-free dry weight basis.

[c]P <0.01

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Table 52. ANOVA of biochemical analyses of control and drilling fluid exposed lobster larvae.

Source of variation		df	SS	MS	F-ratio
Ash[d]					
Stage I	Among groups	2	6.89	3.44	0.33 ns
	CSW and GB-50 vs J-5-50	1	0.20	0.20	0.02 ns
	CSW vs GB-50	1	6.68	6.68	0.79 ns
	Within groups	6	62.34	10.39	-
Stage IV	Among groups (CSW vs J-4-100)	1	0.20	0.20	0.16 ns
	Within groups (CSW vs J-4-100)	4	4.96	1.24	-
Protein[e]					
Stage I	Among groups	2	86.49	43.25	6.77[b]
	CSW and GB-50 vs J-5-50	1	72.53	72.53	10.41[c]
	CSW vs GB-50	1	13.96	13.96	2.31 ns
	Within groups	12	76.61	6.38	-
Stage IV	Among groups (CSW vs J-4-100)	1	6.47	6.47	0.85 ns
	Within groups (CSW vs J-4-100)	6	45.92	7.65	-
Carbohydrate[e]					
Stage I	Among groups	2	0.18	0.09	0.85 ns
	CSW and GB-50 vs J-5-50	1	0.08	0.08	0.79 ns
	CSW vs GB-50	1	0.09	0.09	0.72 ns
	Within groups	12	1.25	0.10	-
Stage IV	Among groups (CSW vs J-4-100)	1	0.13	0.13	1.78 ns
	Within groups (CSW vs J-4-100)	6	0.44	0.07	-
Lipid[e]					
Stage I	Among groups	2	0.48	0.24	0.15 ns
	CSW and GB-50 vs J-5-50	1	0.25	0.25	0.17 ns
	CSW vs GB-50	1	0.22	0.22	0.10 ns
	Within groups	12	19.27	1.61	-
Stage IV	Among groups (CSW vs J-4-100)	1	2.42	2.42	21.46[c]
	Within groups (CSW vs J-4-100)	4	0.45	0.11	-
Protein:Lipid Ratio[a]					
Stage I	Among groups	2.00	0.85	0.42	2.49 ns
	CSW, GB-50 vs J-5-50	1.00	0.85	0.85	5.39[a]
	CSW vs GB-50	1.00	0.0003	0.0003	0.0002 ns
	Within groups	12.00	2.05	0.17	-

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Table 52. (Continued)

Source of variation		df	SS	MS	F-ratio
Stage IV	Among groups (CSW vs J-4-100)	1.00	0.04	0.04	2.50 ns
	Within groups (CSW vs J-4-100)	4.00	0.07	0.02	-

[a]p <0.05
[b]p <0.025
[c]p <0.01
[d]% dry weight basis
[e]% ash-free dry weight basis

Table 53. Barium and calcium analyses of control, barite-500 ppm exposed and J-4 drilling fluid exposed stage I larvae at 96 h.

Group	Ba (µg/mg)	Ca (µg/mg)
CSW	0.0	29.2
Bar-500 ppm	1.4	24.4
J-4-250 ppm	0.0	43.8
J-4-500 ppm	0.0	33.0

[a]Analyses performed by A. Fleer, R. Belastock, P. Brewer of Woods Hole Oceanographic Institution, Woods Hole, Massachusetts.

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SECTION 5

DISCUSSION

Different drilling fluids can vary markedly in their toxicity to larval stages of the lobster, Homarus americanus, as shown in the present study. The 96-h LC_{50} values for the five drilling fluids tested ranged from 74 ppm to greater than 500 ppm. Other investigations on the toxic effects of used, whole drilling fluids and water-soluble fractions of drilling fluids on marine crustaceans (Conklin et al., 1980; Carr et al., 1980; and Page et al., 1980) have also shown wide variations in their toxicity. Conklin et al. (1980) demonstrated a 96-h median lethal concentration of 161 ppm for Mysidopsis with one drilling fluid tested, whereas a concentration of drilling fluid >25,000 ppm was the reported 96-h LC_{50} for Mysidopsis by Carr et al. (1980). The larval stages of lobsters are therefore particularly sensitive to drilling fluid exposure, as has been shown for the early developmental stages of other crustaceans (Atema et al., in press; Neff et al., 1981; Gerber et al., 1980).

Reductions in growth rates, molting frequencies, respiration rates, and feeding rates of larval lobsters following sublethal exposures to certain drilling fluids were also observed in this study. The scope for growth index determined from these physiological measurements indicates a significant degree of stress as a result of drilling fluid exposure. Decreased food consumption was a major contributing factor in the reduced growth efficiencies of lobster larvae exposed to drilling fluids. Lower food consumption may be attributed to numerous variables, such as a weakened condition of the larvae exposed to drilling fluids, interference of chemosensory processes, or change in palatability of the food source. A recent investigation has demonstrated that low concentrations (10-100 ppm) of drilling fluids interfere with the normal physiological response of primary chemosensory cells of adult lobsters to food odors (Derby and Atema, 1981). Lobster larvae fed Artemia nauplii previously contaminated with drilling fluids did not show altered growth or respiration rates except at the highest concentration tested (500 ppm). This would suggest that the palatability of Artemia nauplii was not affected by low-level drilling fluid exposure and is not the major cause of sublethal effects. At the highest concentration tested (500 ppm), lobster larvae fed Artemia contaminated with drilling fluid did show reduced growth rates. Since feeding rates were not measured at this concentration of drilling fluid, the decline in growth rates may have resulted from a change in the nutritional value or palatability of the Artemia.

The reduced respiration rates of the drilling fluid exposed larvae represent an interference of a metabolic process that could result in

inefficient utilization of food and subsequent reductions in growth. Disruption in energy flow, manifested specifically in impairment of growth and development, may be the most serious consequence of drilling fluid exposure. Several investigators have reported similar patterns in the respiration rates and energetics of other marine crustaceans as a result of pollutant exposure (Capuzzo, 1977; Cantelmo et al., 1978). Carr et al. (1980) found that juvenile Mysidopsis had increased respiration rates and decreased growth efficiencies as a result of exposure to 35,000 ppm of the aqueous fraction of a chromium lignosulfonate drilling fluid.

The reported reduction of O:N ratios in drilling fluid exposed lobster larvae is a further indication of an altered physiological state. The O:N ratio has been used as an index of the catabolic balance between protein, carbohydrate and lipid substrates (Bayne, 1975; Capuzzo and Lancaster, 1981) and has been shown to vary under conditions of physiological stress. The reduction in O:N ratios of exposed larvae indicates an increased dependence on protein catabolism for energy production. The ratio of biochemical constituents may provide further information on alterations in energy cycles associated with pollutant stress. Protein and lipids are the major energy substrates utilized by lobsters during larval development (Capuzzo and Lancaster, 1979) and alterations in the protein:lipid ratio were observed with exposure of larval lobsters to South Louisiana crude oil, as a result of interference with lipid metabolism (Capuzzo and Lancaster, 1981). The changes in protein content and the protein:lipid ratio also denote significant shifts in both energy storage and utilization due to drilling fluid exposure. Simultaneous increases in protein catabolism and protein content along with reductions in protein:lipid ratios of drilling fluid exposed stage I larvae suggest that protein is being metabolized for immediate energy needs while less protein is being utilized for de novo synthesis of lipid reserves.

Successful development and metamorphosis of the larval stages of marine crustaceans are dependent on the balance and efficient utilization of energy reserves (Holland, 1978), with lipid reserves being either of primary or secondary importance in the energetics of crustacean development. Alterations in energetics as a result of drilling fluid exposure may impair successful completion of larval development. In addition, the delayed development shown here would also keep the lobsters in a planktonic stage for a longer period, increasing their exposure to predators and other environmental stresses.

Although high concentrations of barite were found in the drilling fluids tested, barium was not detected in the drilling fluid exposed larvae. Barite (500 ppm) exposed lobster larvae had a 0.14 percent barium concentration with these exposure periods. Brannon and Rao (1979) have reported even greater shifts in the total barium concentration of barite exposed Palaemonetes pugio (a benthic-inhabiting shrimp). The level of Ba in whole shrimp exposed to only 50 ppm barite was 1 percent (for controls - 0.003 percent) after a one-week exposure period. This suggests that exposure to the clay components of drilling fluids may become more important to post-larval lobsters that live in the benthos.

The toxicity of used, whole drilling fluids has been shown by other investigators to be primarily due to the soluble chemical constituents (Tagatz, 1980; Sprague and Logan, 1979). The present study includes experiments with barite and a Georges Bank sediment as well as drilling fluids in an effort to isolate physical from chemical factors affecting larval lobsters exposed to drilling fluids. Grain size analyses show that barite has a similar grain size distribution as two of the drilling fluids (MB-5-29 and J-5), but is somewhat coarser than the other drilling fluids (J-4, J-6, and J-7). Georges Bank sediment is coarser than the drilling fluids and the barite. Because barite and Georges Bank sediment had no effect even at very high concentrations (500 ppm) and some of the drilling fluids had an effect on survival, growth, respiration, excretion, O:N ratios, and biochemical composition at comparatively low concentrations (50 ppm), it can be concluded that it is primarily the chemical and not physical features of drilling fluids that are responsible for the harmful effects observed here. It should be noted, however, that histopathological evidence of perturbations in the midgut epithelium has been shown in another crustacean, Palaemonetes pugio, exposed to concentrations of barite comparable to this study (Conklin et al., 1980). It is probable that the chemically based toxicity demonstrated with these drilling fluids acts in combination with such perturbations as those resulting from the physical properties of the clays in drilling fluid.

Whole used drilling fluids were used in this study in order to simulate as closely as possible the chemicals that lobsters will be exposed to with ocean discharges. There are two factors that make it difficult to identify which classes of constituents are primarily responsible for the toxicity of each drilling fluid used in this study. The chemical compositions of the drilling fluids are incompletely known and there is the possibility of synergistic interactions among the many constituents. Nevertheless, certain chemicals that are known to be toxic to some marine crustaceans are known to be present in the drilling fluids studied here; these chemicals include petroleum products, metals, and phenols (Capuzzo and Lancaster, 1981; Wells and Sprague, 1976; Brannon and Rao, 1979; Cantelmo et al., 1978).

J-4 was the least toxic drilling fluid of all tested in terms of the 96-h LC_{50} values and of all the physiological parameters measured. This drilling fluid had a lower barium, chromium, zinc, phenol, and diesel content than the other J-drilling fluids tested. The most toxic J-drilling fluid, J-5, had a lower percent Diesel content than J-6 or J-7; however, chromium and phenol levels were higher in this drilling fluid. MB-5-29 also had a relatively low Diesel content and considerably higher chromium and phenol concentrations as compared to the J-drilling fluids. MB-5-29 exposed stage I larvae had a 96-h LC_{50} value closely comparable to J-6 exposed larvae; sublethal effects resulting from MB-5-29 exposures were seen at a concentration as low as 10 ppm. Thus, direct correlations between percent Diesel and relative toxicity cannot be made, although the diesel oil was apparently an important contributing factor. The biochemical changes that occurred with drilling fluid exposure parallel findings reported on oil-exposed larval lobsters (Capuzzo and Lancaster, 1981). Oil-exposed larvae also had significantly reduced O:N ratios as was shown for the larvae exposed to drilling fluids with high hydrocarbon contents.

The likelihood that lobster larvae in their natural environment will encounter concentrations of drilling fluids comparable to those used in these experiments is an important question. Dispersion rates and distribution patterns of drilling fluids released into oceanic waters will depend on the specific location of the drilling site in terms of local atmospheric and hydrographic conditions. Studies have been conducted on the distribution and concentration of discharged drilling fluids in the vicinity of drill sites (Dames and Moore, 1978; Ayers et al., 1980; Gettleson and Laird, 1980). The parameters measured by these studies in an effort to trace the drilling fluid plumes include concentrations of suspended solids and of barium. Ayers et al. (1980) conducted an on-site dispersion study in the mid-Atlantic Ocean during a bulk mud discharge (275 barrel/h). A suspended solids concentration of 122 ppm was measured at 15 m from the discharge pipe, 11 times lower than at zero meters. Background suspended solid levels were reached at approximately 600 m from the source. Gettleson and Laird (1980) conducted barium analyses on sediment samples in the vicinity of drilling sites in the Gulf of Mexico. Their results indicate that drilling fluids can be dispersed in detectable quantities at least 1,000 meters from the source. The results of these field monitoring studies, however, provide no answer to questions concerning the fate of the soluble fractions of drill fluids. The present study has shown that these fractions are of the greatest concern in terms of causing detrimental effects to the early life stages of a marine organism. Since it is likely that the soluble fractions are dispersed further from the drilling sites than the heavier fractions, the area of exposure may actually be much larger than that indicated in the above studies.

REFERENCES

Atema, J., D. F. Leavitt, D. E. Barshaw, and M. C. Cuomo. (In press). Water column and substrate effects of drilling muds on behavior of juvenile lobsters, Homarus americanus. Can. J. Fish. Aquat. Sci.

Ayers, R. C., T. C. Sauer, R. P. Meek, and G. Bowers. 1980. An environmental study to assess the impact of drilling discharges in the mid-Atlantic. In: Water Quality Monitoring, Mid-Atlantic Outer Continental Shelf Area, EG and G, Environmental Consultants, p. 7-58.

Bayne, B. L. 1975. Aspects of physiological condition in Mytilus edulis L., with special reference to the effects of oxygen tension and salinity. Proc. Europ. Mar. Biol. Symp. IX: 213-238.

Bligh, E. G. and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37: 911-917.

Brannon, A. C. and K. R. Rao. 1979. Barium, strontium and calcium levels in the exoskeleton, hepatopancreas and abdominal muscle of the grass shrimp Palaemonetes pugio: Relation to molting and exposure to barite. Comp. Biochem. Physiol., 63A: 261-274.

Cabrera, J. 1971. Survival of the oyster Crassostrea virginica (Gmelin) in the laboratory under the effects of oil drilling fluids spilled in the Laguna de Tamiahua, Mexico. Gulf Research Reports 3: 197-213.

Cantelmo, A. C., P. J. Conklin, F. R. Fox, and K. R. Rao. 1978. Effects of Na-PCP and 2,4-DNP on respiration in crustaceans. In: K. R. Rao (ed.), Pentaclorophenol, Plenum Press, New York, pp. 251-264.

Capuzzo, J. M. 1977. The effects of free chlorine and chloramine on growth and respiration rates of larval lobsters (Homarus americanus). Water Res. 11: 1021-1024.

Capuzzo, J. M., S. A. Lawrence and J. A. Davidson. 1976. Combined toxicity of free chlorine, chloramine and temperature to stage I larvae of the American lobster Homarus americanus. Water Res. 10: 1093-1099.

Capuzzo, J. M. and B. A. Lancaster. 1979. Some physiological and biochemical considerations of larval development in the American lobster, Homarus americanus Milne Edwards. J. Exp. Mar. Biol. Ecol. 40: 53-62.

Capuzzo, J. M. and B. A. Lancaster. 1981. The physiological effects of South Louisiana crude oil on larvae of the American lobster (Homarus americanus). In: F. J. Vernberg, A. Calabrese, F. P. Thurberg and W. B. Vernberg (eds.), Biological Monitoring of Marine Pollutants, Academic Press, New York, pp. 405-423.

Carr, R. S., L. A. Reitsoma, J. M. Neff. 1980. Influence of a used chrome lignosulfonate drilling mud on the survival, respiration, feeding activity and net growth efficiency of the opossum shrimp, Mysidopsis almyra. Sym. Res. Environ. Fate and Effects of Drilling Fluids and Cuttings II: 944-960.

Chesser, B. G. and W. H. McKenzie. 1975. Use of bioassay test in evaluating the toxicity of drilling fluid additives on Galveston Bay shrimp. In: Environmental Aspects of Chemical Use in Well-Drilling Operations. U.S. Environmental Protection Agency, Washington, DC, EPA-560/1-75-004.

Chow, T. J. 1976. Barium in Southern California coastal waters: a potential indicator of marine drilling contamination. Science 193: 57-58.

Chow, T. J., J. L. Earl, J. H. Reed, N. Hansen and V. Orphan. 1978. Barium content of marine sediments near drilling sites: a potential pollutant indicator. Mar. Pollut. Bull. 9: 97-99.

Cobb, S. J. 1976. The American lobster: the biology of Homarus americanus. University of Rhode Island Sea Grant Tech. Rep., Kingston, RI 32 pp.

Conklin, P. J., D. G. Doughtie, and K. Ranga Rao. 1980. Effects of barite and used drilling muds on crustaceans, with particular reference to the grass shrimp, Palaemonetes pugio. Sym. Res. Environ. Fate and Effects of Drilling Fluids and Cuttings. II: 912-940.

Dames and Moore. 1978. Drilling fluid dispersion and biological effects study for the Lower Cook Inlet C.O.S.T. well. A report for Shell Oil Company.

Daugherty, F. M. 1951. Effects of some chemicals used in oil well drilling on marine animals. Sew. Ind. Wastes 23: 1282-1287.

Derby, C. D. and J. Atema. 1981. Influence of drilling muds on the primary chemosensory neurons in walking legs of the lobster, Homarus americanus. Can. J. Fish. Aquat. Sci. 38: 268-274.

EIFAC. 1965. Water quality criteria for European freshwater fish. Report on finely divided solids and inland fisheries. Air Water Pollut. 9: 151-168.

Finney, D. J. 1971. Probit analysis. Cambridge University Press, Cambridge. 333 pp.

Folch, J., M. Lees and G. H. S. Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226: 497-509.

George, R. Y. 1975. Potential effects of oil drilling and dumping activities on marine biota. In: Environmental Aspects of Chemical Use in Well-Drilling Operations. U.S. Environmental Protection Agency, Washington, DC, EPA-560/1-75-004, pp. 333-355.

Gerber, R. P., E. S. Gilfillan, B. T. Page, D. S. Page, J. B. Hotham. 1980. Short and long term effects of used drilling fluids on marine organisms. Sym. Res. Environ. Fate and Effects of Drilling Fluids and Cuttings II: 882-909.

Gettleson, D. A. and C. E. Laird. 1980. Benthic barium levels in the vicinity of six drill sites in the Gulf of Mexico. Sym. Res. Environ. Fate and Effects of Drilling Fluids and Cuttings II. 739-785.

Herrick, F. H. 1896. The American lobster: A study of its habits and development. Bull. U.S. Fish. Comm. 15: 1-252.

Holland, D. L. 1978. Lipid reserves and energy metabolism in the larvae of benthic marine invertebrates. In: D. C. Malins and J. R. Sargent (eds.), Biochemical and Biophysical Perspectives in Marine Biology, Vol. 4, Academic Press, New York, pp. 85-123.

Holland, D. L. and P. A. Gabbott. 1971. A micro-analytical scheme for the determination of protein, carbohydrate, lipid, and RNA levels in marine invertebrate larvae. J. Mar. Biol. Ass. U.K. 51: 659-668.

Hughes, J. T. and G. C. Matthiessen. 1962. Observations on the biology of the American lobster, Homarus americanus. Limnol. Oceanogr. 7: 414-421.

Hughes, J. T., R. A. Shleser and G. Tchobanoglous. 1974. A rearing tank for lobster larvae and other aquatic species. Prog. Fish. Cult. 36: 129-132.

Land, B. 1974. The toxicity of drilling fluid components to aquatic biological systems, a literature review. Tech. Rep. No. 487, Research and Dev. Directorate, Freshwater Inst., Manitoba 33 pp.

Logan, D. T. and C. E. Epifanio. 1978. A laboratory energy balance for the larvae and juveniles of the American lobster, Homarus americanus. Mar. Biol. 47: 381-389.

Neff, J. M., R. S. Carr and W. L. McCulloch. 1981. Acute toxicity of a used chrome lignosulfonate drilling mud to several species of marine invertebrates. Mar. Environ. Res. 4: 251-266.

Page, D. S., B. T. Page, J. R. Hotham, E. S. Gilfillan and R. P. Gerber. 1980. Bioavailability of toxic constituents of used drilling muds. Sym. Res. Environ. Fate and Effects of Drilling Fluids and Cuttings II: 984-993.

Rao, K. R., F. R. Fox, P. J. Conklin, A. C. Cantelmo and A. C. Brannon. 1979. Physiological and biochemical investigations of the toxicity of pentachlorophenol to crustaceans. In: W. B. Vernberg, A. Calabrese, F. P. Thurberg and F. J. Vernberg (eds.), Marine Pollution: Functional Responses. Academic Press, New York. pp. 307-339.

Raymont, J. E. G., J. Austin and E. Linford. 1964. Biochemical studies on marine zooplankton. I. The biochemical composition of Neomysis integer. J. Cons. Perm. Int. Explor. Mer 28: 354-363.

Richards, N. L. 1979. Effects of chemicals used in oil and gas well-drilling operations in aquatic environments. In: Fourth National Conference, Interagency Energy/Environment Research and Development Program. U.S. Environmental Protection Agency, Office of Energy, Minerals and Industry, Washington, DC, pp. 1-12.

Sokal, R. R. and F. J. Rohlf. 1969. Biometry. W. H. Freeman Co., San Francisco, CA. 776 pp.

Solorzano, L. 1969. Determination of ammonia in natural waters by the phenolhypochorite method. Limnol. Oceanogr. 14: 799-801.

Spencer, D. W., P. G. Brewer, A. Fleer, S. Honjo, S. Krishnaswami and Y. Nozaki. 1978. Chemical fluxes from a sediment trap experiment in the deep Sargasso Sea. J. Mar. Res. 36: 493-523.

Sprague, J. B. 1969. Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. Water Res. 3: 793-821.

Sprague, J. B. and W. J. Logan. 1979. Separate and joint toxicity to rainbow trout of substances used in drilling fluids for oil exploration. Environ. Pollut. 19: 269-281.

Tagatz, M. E., J. M. Ivey, H. K. Lehman, M. Tobia and J. L. Ogelsby. 1980. Effects of drilling mud on development of experimental estuarine macrobenthic communities. Sym. Res. Environ. Fate and Effects of Drilling Fluids and Cuttings II: 847-861.

Thompson, J. H., Jr. and T. J. Bright. 1977. Effects of drill mud on sedimentary clearing rates of certain hermatypic corals. Proc. 1977 Oil Spill Conf. American Petroleum Inst. Publ No. 4284: 495-498.

van Kley, H. and S. Hale. 1977. Assay for protein by dye binding. Anal. Biochem. 81: 485-486.

Wells, P. G. and J. B. Sprague. 1976. Effects of crude oil on American lobster (Homarus americanus) larvae in the labortory. J. Fish. Res. Bd. Canada 33: 1604-1614.